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# CONTENTS OF VOLUME 41

JANUARY 1946. NUMBER 1

PAGE

Absorption of Scar Tissue in Experimental Nodular Cirrhosis of the Liver, with a Method of Visualizing Cirrhotic Changes. Bernhard Steinberg, M.D., and Ruth A. Martin, M.T., Toledo, Ohio.....	1
Microscopic Lesions in Acetylcholine Shock. Rudolf Altschul, M.U.Dr., and M. M. Laskin, B.A., Saskatoon, Saskatchewan, Canada.....	11
Relation of Cholelithiasis to Acute Hemorrhagic Pancreatitis. D. W. Molander, B.S., and E. T. Bell, M.D., Minneapolis.....	17
Experimental Pituitary Diabetes of Five Years' Duration with Glomerulosclerosis. F. D. W. Lukens, M.D., and F. C. Dohan, M.D., Philadelphia.	19
Morphologic Studies of Rats Deprived of Essential Amino Acids: III. Histidine. Mark E. Maun, M.D.; William M. Cahill, Ph.D., and Ruth M. Davis, B.A., Detroit.....	25
Occurrence of Rheumatic Carditis in the Native Population of Curaçao, Netherlands West Indies. Philip H. Hartz, M.D., and Ary van der Sar, M.D., Curaçao, Netherlands West Indies.....	32
Fat Necrosis Studies: VI. The Effect of Feeding Lipase-Containing Vegetable Seed on the Production of Fat Necrosis. M. Pinson Neal, M.D., Columbia, Mo.....	37
Experimental Nephropathies: IV. Glycosuria in Dogs Poisoned with Uranyl Nitrate, Mercury Bichloride and Potassium Dichromate. Opal E. Hepler, M.D., and J. P. Simonds, M.D., Chicago.....	42
Leukopenia and Inflammation: The Presence of a Leukopenic Factor in Inflammatory Exudates. Valy Menkin, M.D., Durham, N. C.....	50
<b>Case Reports:</b>	
Obstruction of the Aortic Isthmus by a Calcified Thrombus. Herbert D. Axilrod, M.D., New Haven, Conn.....	63
<b>General Reviews:</b>	
Tropical Diseases: Involvement of the Nervous System. W. S. Chalgren, M.D., and A. B. Baker, M.D., Minneapolis.....	66
Book Reviews.....	118
Notes and News.....	120

FEBRUARY 1946. NUMBER 2

Distribution of Lipase in the Tissues Under Normal and Under Pathologic Conditions. George Gomori, M.D., Ph.D., Chicago.....	121
Experimental Studies in Cardiovascular Pathology. W. C. Hueper, M.D., New York:	
XII. Atheromatosis in Dogs Following Repeated Intravenous Injections of Solutions of Hydroxyethylcellulose.....	130
XIII. Vibratory Lability of Plasma Colloids in Rabbits and in Dogs Following Ingestion of Cholesterol.....	139

## FEBRUARY—Continued

	PAGE
Structural Changes in the Thyroid Glands of Patients Treated with Thiouracil. Béla Halpert, M.D.; John W. Cavanaugh, M.D., and Bert F. Keltz, M.D., Oklahoma City.....	155
Sclerema Adiposum Neonatorum of Both Internal and External Adipose Tissue. Pearl Zeek, M.D., and Ethel Mae Madden, M.D., Cincinnati.....	166
"Alveolar Cell Tumor" of the Lung: Further Evidence of Its Bronchiolar Origin. Peter A. Herbut, M.D., Philadelphia.....	175
Experimental Nephropathies: VI. The Problem of Experimental Glomerulo- nephritis. James P. Simonds, M.D.; Herman J. Linn, M.D., and Jack Lange, M.D., Chicago.....	185
Pituitary Lesions Accompanying Obesity. Joseph W. Goldzieher, M.D., New York.....	203
<b>Case Reports:</b>	
Adenoacanthoma of the Stomach. George Strassmann, M.D., Waltham, Mass. ....	213
Notes and News.....	220
Book Reviews.....	221

## MARCH 1946. NUMBER 3

Diagnosis of Erythroblastosis (Hemolytic Anemia) in the Macerated Fetus. Edith L. Potter, M.D., Ph.D., Chicago.....	223
Hypertension and Necrotizing Arteritis in the Rat Following Renal Infarction. Dorothy Loomis, M.D., Brooklyn.....	231
An Analysis of the Klippel-Feil Syndrome. C. A. Erskine, M.R.C.P.I., New- castle Upon Tyne, England.....	269
Ovarian Involvement in Hodgkin's Disease. Elwyn L. Heller, M.D., and William Palin, M.D., Pittsburgh.....	282
Plasmodium Falciparum Malaria: The Coronary and Myocardial Lesions Observed at Autopsy in Two Cases of Acute Fulminating P. Falciparum Infection. Lieutenant Colonel Walter C. Merkel, Medical Corps, Army of the United States.....	290
Reticulum. William B. Dublin, M.D., Indianapolis.....	299
Role of Stasis in the Development of Pulmonary Infarcts. Campbell Moses, M.D., Pittsburgh.....	319
<b>Case Reports:</b>	
Intestinal Perforation in Paratyphoid Due to <i>Salmonella Paratyphi B.</i> Captain Irving Zeidman and Major Charles C. Randall, Medical Corps, Army of the United States.....	322
An Acute Febrile Illness Characterized by Thrombopenic Purpura, Hemo- lytic Anemia and Generalized Platelet Thrombosis. Frank E. Trobaugh Jr., M.D.; Martin Markowitz, M.D., and Charles S. Davidson, M.D., Boston, and Walter F. Crowley, M.D., Franklin, Mass. .....	327
Epidermoid Carcinoma Arising in an Endometrial Cyst of the Ovary. Kendrick McCullough, M.D., and Esley R. Froats, M.D., Yonkers, N. Y., and Henry C. Falk, M.D., New York.....	335

## MARCH—Continued

	PAGE
Obituaries:	
Joseph McFarland, M.D.....	338
Notes and News.....	341
Books Received.....	342
Book Reviews.....	343

## APRIL 1946. NUMBER 4

Pathologic Aspects of Acute Epidemic Hepatitis, with Especial Reference to Early Stages: Report of a Series of Ten Cases, Including a Case in Which There Was Spontaneous Rupture of the Spleen and Six Cases of Fulminating Disease in Patients Who Had Been Wounded Several Months Previously. Commander David A. Wood, MC(S), U.S.N.R....	345
Chemical Factors and Their Role in Inflammation. Valy Menkin, M.D., Durham, N. C.....	376
Adenocarcinoma of the Urachus Involving the Urinary Bladder. Lieutenant Colonel Arthur E. Rapoport and Lieutenant Colonel Charles E. Nixon, Medical Corps, Army of the United States.....	388
Teratoma of the Anterior Mediastinum in the Group of Military Age: A Study of Sixteen Cases, and a Review of Theories of Genesis. Captain Hans G. Schlumberger, Medical Corps, Army of the United States.....	398
Case Reports:	
Struma Ovarii. Elwyn L. Heller, M.D., and Luther Spoehr, M.D., Pittsburgh .....	445
Hepatic Abscess Complicating Atresia of the Small Intestine of a Newborn Infant. Sumner Price, M.D., and Thomas Chang, M.D., Honolulu, Territory of Hawaii.....	450
Notes and News.....	454
Books Received.....	456

## MAY 1946. NUMBER 5

Changes in the Central Incisors of Hypophysectomized Female Rats After Different Postoperative Periods. Hermann Becks, M.D., D.D.S., and Daniel A. Collins, D.D.S., San Francisco, and Miriam E. Simpson, M.D., and Herbert M. Evans, M.D., Berkeley, Calif.....	457
Renal Lesions in Portal Cirrhosis. J. H. Baxter, M.D., and C. T. Ashworth, M.D., Dallas, Texas.....	476
Transitory Pulmonary Infiltrations (Loeffler's Syndrome) in Rabbits. Peter A. Herbut, M.D., and Frank R. Kinsey, M.D., Philadelphia.....	489
Adrenal Hemorrhages in Meningococcic Sepsis. J. Schwarz, M.D., Valdivia, Chile .....	503
Radioiodine Autography in Studies of Human Goitrous Thyroid Glands. C. P. Leblond; M. Been Fertman; I. D. Poppel, M.D., and George M. Curtis, M.D., Columbus, Ohio.....	510
Hepatic and Renal Necrosis in Alloxan Diabetes of Rabbits. Peter A. Herbut, M.D.; John S. Watson, M.D., and Ella Perkins, M.Sc., Philadelphia....	516
Heart Weight: II. The Effect of Tuberculosis on Heart Weight. Pearl M. Zeek, M.D., Cincinnati.....	526
Genesis of Aortic Perforation Secondary to Carcinoma of the Esophagus: Report of Observations in Two Cases. A. V. Postoloff, M.D., Buffalo, and W. M. Cannon, M.D., Charleston, S. C.....	533

## MAY—Continued

PAGE

## Case Reports:

Enteritis Caused by <i>Salmonella</i> <i>Suispestifer</i> with Secondary Moniliasis. George E. Gutmann, M.D., Boston.....	540
Primary Carcinoma of the Liver of a Dog. Walter M. Booker, Ph.D., and A. C. Webb, M.D., Washington, D. C.....	548
Hemopericardium from Pericardial Metastatic Carcinoma. George J. Rukstnat, M.D., Chicago.....	550
Teratoma of Pineal Gland with Choriocarcinoma and Rhabdomyosarcoma. Robert L. Glass, M.D., and C. G. Culbertson, M.D., Indianapolis....	552

## Laboratory Methods and Technical Notes:

A Portable Exhibit Case. George J. Rukstnat, M.D., Chicago.....	556
Improved Methods for Demonstrating Amyloid in Paraffin Sections. Benjamin Highman, M.D., Bethesda, Md.....	559

## Notes and News.....

## Books Received.....

## JUNE 1946. NUMBER 6

Local Tissue Reactivity (Shwartzman Phenomenon) in the Heart and the  
Femoral Artery of the Rabbit. C. G. Tedeschi, M.D., Framingham, Mass. 565Chorionic Gonadotropin in the Diagnosis of Testicular Tumors. John I.  
Brewer, M.D., Ph.D., Chicago.....Toxicity and Detoxication of Cinchophen: Experimental Studies. W. C.  
Hueper, M.D., New York.....Human Strongyloidiasis with Internal Autoinfection. Philip H. Hartz, M.D.,  
Curaçao, Netherlands West Indies.....Tropical Ulcer in Guatemala: Pathologic, Bacteriologic, Mycologic and Clinical  
Aspects. Major Alfred Golden, Medical Corps, Army of the United  
States, and Enrique Padilla B., M.D., M.P.H., Guatemala.....Ethylene Glycol Poisoning, with Suggestions for Its Treatment as Oxalate  
Poisoning. Commander George Milles (MC), U. S. N. R.....Etiologic Concepts and Pathologic Aspects of Ainhum. Major B. H. Kean,  
Medical Corps, Army of the United States, and Harold A. Tucker, M.D.,  
Baltimore .....Primary Cystic Tumor of the Diaphragm. Orland B. Scott, M.D., and  
Douglas R. Morton, M.D., Chicago.....Development of Sebaceous Glass, from Intralobular Ducts of the Parotid  
Gland. Philip H. Hartz, M.D., Curaçao, Netherlands West Indies.....

## Case Reports:

Leiomyosarcoma Involving the Right Ureter. A. X. Rossien, M.D., and Thomas H. Russell, M.D., New York.....	655
---	-----

Cryptothroid Adenoma, with Uremia Due to Calcification of the Kidneys. Paul Lober, M.D.; Ambrose J. Herzog, M.D., and Carl O. Rice, M.D., Minneapolis.....	661
--	-----

Splenic Hemangiosarcoma: A Case with Lymphatic and Vascular Meta- stases. Donald deF. Bauer, M.D., and W. Raney Stanford, M.D., Durham, N. C.....	668
---	-----

## Laboratory Methods and Technical Notes:

A Rapid Gram Stain for Tissue. Edwin M. Lerner II, M.D., Boston....	674
A Hemoglobin Stain for Histologic Use Based on the Cyanol-Hemoglobin Reaction. R. C. Dunn, M.D., Bethesda, Md.....	676

## Notes and News.....

## General Index.....

679

# ARCHIVES OF PATHOLOGY

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COPYRIGHT, 1946, BY THE AMERICAN MEDICAL ASSOCIATION

## ABSORPTION OF SCAR TISSUE IN EXPERIMENTAL NODULAR CIRRHOSIS OF THE LIVER

*With a Method of Visualizing Cirrhotic Changes*

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IT IS generally accepted that the fibrosis of the cirrhotic liver is an irreversible change. It is also believed that no structural repair is possible after scar tissue has been laid down and the hepatic parenchyma subdivided haphazardly. The causes of most forms of cirrhosis in which scarring is conspicuous are not well understood, and treatment is ineffectual. The condition progresses unfavorably and terminates in death. Hence in the human type of the disease the concept of "irrevocable change" cannot be tested. The "nodular cirrhosis" produced with azo compounds by Yoshida,<sup>1</sup> Nakazawa<sup>2</sup> and Kinoshita<sup>3</sup> offered an opportunity to verify whether scarring, at least in the experimental form of portal cirrhosis, is irreversible.

Orr<sup>4</sup> produced nodular cirrhosis in rats by feeding them 20 to 30 cc. of 3 per cent butter yellow (paradimethylaminoazobenzene) in olive oil per kilogram of wheat or unpolished rice, with a supplement of "green stuff." The investigator terminated the feeding of butter yellow at the end of the seventh month. He killed a number of animals monthly for a period of eleven months. He presents an excellent description of the progressive changes of cirrhosis. One must be critical, however, of his assumption that cirrhosis begins in all animals at the same time and in the same degree.<sup>5</sup> Orr also noted that in many of the rats the structural pattern of the liver returned to normal after the tenth month. Although this observation suggests reversibility of the fibrotic process, it is by no means conclusive. Random selection of animals each month presupposes that the disease develops in all of them in equal degree. This supposition does not coincide with the results of our experiments.

From the Toledo Hospital Institute of Medical Research.

1. Yoshida, T.: Gann **29**:213, 1935.
2. Nakazawa, T.: Tr. Soc. path. jap. **26**:613, 1936.
3. Kinoshita, R.: Tr. Soc. path. jap. **27**:665, 1937.
4. Orr, J. W.: J. Path. & Bact. **50**:393, 1940.

In our preliminary investigations we noted the presence of normal hepatic patterns in some animals after butter yellow was withdrawn. However, the livers of other rats which died or were killed while restricted to the diet either showed patchy change or were completely normal. These observations indicated that the presence and the degree of cirrhosis must be ascertained in the living animals prior to investigations dealing with recovery. A procedure which satisfied these requirements was described by us in collaboration with Walliker.<sup>5</sup> A colloidal suspension of thorium dioxide was introduced intracardially in rats. The progressive cirrhotic changes could be readily visualized on roentgenograms.

#### EXPERIMENTAL PROCEDURES

Fifty albino rats of the Wistar strain, 3 to 5 months of age and of both sexes, were used in the experiment. The animals were given a diet of brown unpolished ground rice with butter yellow in the concentration of 0.06 per cent and 1 Gm. of carrot daily. The ration was prepared by dissolving 3 Gm. of butter yellow in 100 cc. of olive oil with gentle heating. Each 1,000 Gm. of rice was thoroughly mixed with 20 cc. of the butter yellow solution. No limitation was placed on the quantity of butter yellow-rice mixture consumed by the animals. However, their consumption of carrots was restricted. The feeding of unlimited amounts of vegetables is inadvisable for several reasons. For one, the animals consume the vegetables and do not eat the butter yellow-rice mixture.

The colloidal suspension of thorium dioxide was injected intracardially in 0.5 cc. quantities from two to four times. The first two injections were made at an interval of three months and the others after the animal was returned to its normal diet. Roentgenograms were taken of chest and abdomen prior to the feeding of butter yellow and at monthly intervals thereafter for the duration of the animal's life. Visualization of the liver and the spleen was obtained on roentgenograms as follows: Projection was anteroposterior and posteroanterior. No filter was used. The distance was 36 in., the kilovolt peak 60 and the amperage 100 ma. An Eastman no-screen film was used. The animals were under ether anesthesia.

A group of 26 animals was used in a study of the correlation of gross and microscopic changes of the liver with the roentgenographic appearances. Rats were killed at intervals of from two weeks to seven months after the start of the dietary regimen.

A group of 24 rats was allowed to acquire various degrees of cirrhosis. When the presence and the extent of the cirrhosis had been established by roentgenograms, the animals were returned to a stock diet made up as follows: cornmeal 69.64 per cent, casein 3.08 per cent, soybean oil meal 10.8 per cent, alfalfa leaf meal 2.06 per cent, wheat germ 10.8 per cent, yeast 2.06 per cent, calcium carbonate 0.52 per cent, cod liver oil 0.52 per cent and salt 0.52 per cent. At intervals of from two weeks to eight and a half months after the return to the stock ration, 2 to 4 animals were killed. The presence of cirrhosis and the degree of return to normal as determined by gross and microscopic criteria were correlated with the roentgenographic appearances.

5. Steinberg, B.; Walliker, C. T., and Martin, R. A.: Proc. Soc. Exper. Biol. & Med. 55:165, 1944.

CORRELATION OF ROENTGENOGRAPHIC AND  
PATHOLOGIC OBSERVATIONS

The normal liver was visualized roentgenographically as a diffuse shadow. Within the first two months of the butter yellow-rice-carrot diet the roentgenograms of some animals showed a patchy concentration of thorium dioxide and the appearance of poorly defined "ring shadows." After two months of the diet the roentgenograms of a few of the rats showed fairly well defined ring shadows with thick walls alternating with areas of diffuse shadow. From the third month on, the ring shadows became more numerous and entirely replaced the diffuse shadow. The ring shadows were for the most part uniform in size. In some areas they were fairly large.

*TABLE 1.—Correlation of Gross and Microscopic Changes in the Liver with Roentgenographic Appearances After Variable Periods of Butter Yellow-Rice-Carrot Diet*

Rats	Duration of Diet	Pathologic Changes	Roentgenographic Appearance
2	2 wk.	Fatty metamorphosis	No changes
5	5 wk.	Diffuse cellular degenerative changes	No changes
5	2 mo.	Degenerative cellular changes to moderate fibrosis	No change in some; appearance of concentration of thorium dioxide and poorly defined ring shadows—cirrhotic configuration—in others
3	3 mo.	Degenerative cellular changes to moderate fibrosis	Distinct ring shadows—slight to moderate cirrhotic configuration
5	4 mo.	Moderate to considerable cirrhosis	Moderate to marked cirrhotic configuration
6	5 to 7 mo.	No cirrhosis in 1 rat; slight to marked cirrhosis in others; new growths in some animals	No cirrhosis in 1 rat; slight to marked cirrhotic configuration in others; new growth outlined in some animals

Histologic studies of liver tissue in an early stage of cirrhosis and prior to the appearance of connective tissue showed thorium dioxide within vessels of portal spaces and in sinusoidal capillaries. The homogeneous shadow of the roentgenogram was due to the diffuse spread of thorium dioxide in the sinusoidal capillary system. A part of the thorium dioxide was phagocytosed by large mononuclear cells, and the remainder was free. In those livers with considerable deposition of connective tissue, representing advanced cirrhosis, thorium dioxide became localized to the vessels of the perilobular and intralobular connective tissue. Thorium dioxide was present also in relatively large quantities in arteries of the portal spaces. There was little if any of the material in the sinusoidal capillaries of hepatic nodules surrounded by scar tissue. The ring shadows apparently represented connective tissue with concentration of thorium dioxide. The clear areas were the circumscribed

nodules of liver. Degenerative changes of the cells did not alter the roentgenographic shadow.

With advanced cirrhosis, evidenced by extensive and diffuse nodularity, the ring shadows occupied the entire liver. The degree and the extent of cirrhosis could be determined on the basis of the size, the number and the distribution of the ring shadows in the lobes of the liver (fig. 1).

By neither of the criteria, roentgenographic shadow or pathologic change, was there any uniformity of development or of degree of cirrhosis. Although all the animals were given the diet at the same time and were maintained in the same environment of controlled temperature and humidity, there was considerable variation in the appearance of the livers. Some of the rats failed to show cirrhosis after four to six months of butter yellow diet. Other animals showed extensive cirrhosis after two months of the diet. There was also considerable difference in various areas of the same liver. Some parts of the liver had extensive perilobular and intralobular fibrosis, with or without cellular degeneration, while in other areas the tissue appeared normal and without structural distortion. It was possible to identify all the degenerative and proliferative changes of cirrhosis in the same liver (fig. 2).

#### EFFECT OF RETURN TO NORMAL DIET ON CIRRHOTIC LIVERS

In six weeks after the animals were returned to their normal diet, roentgenographic changes became apparent in most of the animals. The shadow rings became larger and the walls thinner. In some areas the shadow rings became either indistinct or disappeared entirely. Histologically there was a reduction of connective tissue. Thorium dioxide was present in small quantities in the remaining connective tissue. It appeared to be concentrated in the vessels of the portal spaces.

In the subsequent periods up to the fifth month the hepatic changes were progressive but not significant. The shadows tended to become diffuse. In patchy areas the rings were reduced in number and increased in size. After the fifth month some of the animals showed complete return to a normal roentgenographic picture. In other rats the improvement varied from slight to moderate. New growths appeared in the livers of most of the animals. Appearance of and degree of recovery were not consistent in the animals. There was considerable variation among the livers of the rats as well as within the liver of the same animal. Histologic examination of one or two areas of a liver did not give a true picture of the whole organ. Gross study of slabs of liver with microscopic correlations of multiple grossly differing areas gave a truer evaluation (table 2 and fig. 3).



Fig. 1.—Correlation of roentgenographic and histologic changes of livers of rats fed a butter yellow-rice-carrot diet.

*A*, roentgenogram of a rat which had been maintained on the butter yellow-rice-carrot diet for one month and in which a colloidal suspension of thorium dioxide was then injected intracardially. The liver and the spleen are visualized as diffuse shadows, a roentgenographic appearance suggestive of normal organs.

*B*, section of the liver of the rat in *A*. Note the diffuse presence of thorium dioxide in the sinusoidal capillaries and in the vessels of the portal spaces. There are degenerative and congestive changes but no fibrosis. The diffuse distribution of the thorium dioxide is responsible for the homogeneous shadows.

*C*, roentgenogram of a rat fed the butter yellow-rice-carrot diet for two months, with thorium dioxide showing cirrhotic configuration. Note the ring shadows due to thorium dioxide present in the connective tissue.

*D*, section of the liver of the rat in *B*. Note the localization of thorium dioxide in the connective tissue around nodules of liver tissue which results in ring shadows.

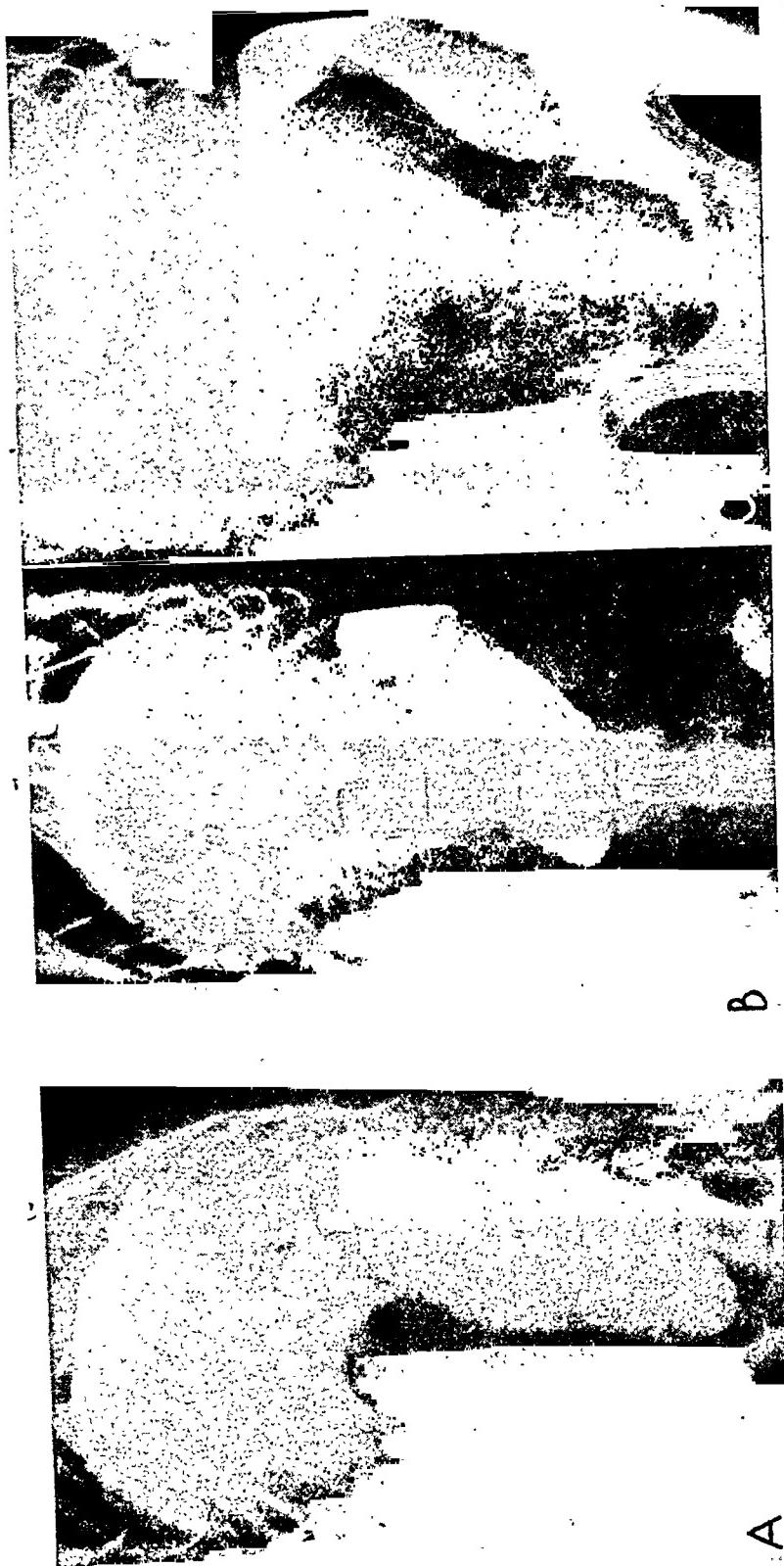


Fig. 2.—Roentgenograms of 3 rats which had been fed a butter-yellow-rice-carrot diet for four months and in which a colloidal suspension of thorium dioxide was then injected intracardially. These roentgenograms show variable degrees of hepatic cirrhosis after the same period of butter yellow diet.  
*A*, diffuse shadow indicating that no scarring has occurred. The roentgenogram is similar to that of a normal animal.  
*B*, indistinct but fairly large ring shadows without clearcut walls, localized to a few areas. There is concentration of thorium dioxide in some areas. Persistence of diffuse shadow indicates an early stage of cirrhosis, with a small amount of connective tissue.  
*C*, extensive presence of small ring shadows with fairly thick walls throughout the liver. This appearance is indicative of a fairly advanced diffuse cirrhosis.

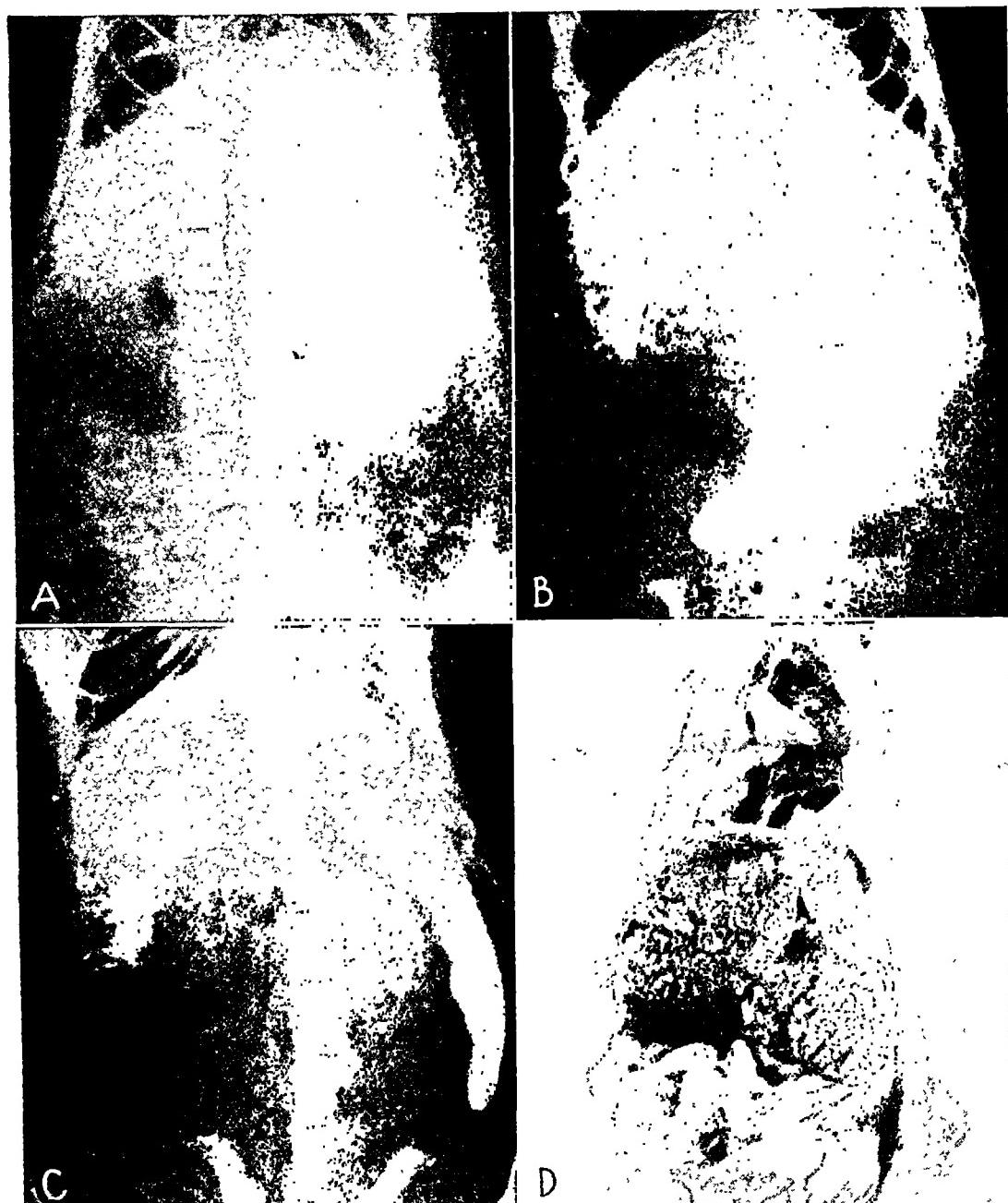


Fig. 3.—Roentgenograms and gross appearance of a rat fed butter yellow for four months and the normal stock ration for six weeks. *A*, roentgenogram before feeding of butter yellow. *B*, roentgenogram after four months of butter yellow-rice-carrot diet. Note the diffuse ring shadows. *C*, roentgenogram six weeks after return to the normal stock ration. Note the tendency to return to a diffuse shadow, the increase in the size of the ring shadows and the hazy ring walls.

*D*, gross photograph of the liver of the animal shown in *A*, *B* and *C*, taken six weeks after return to the normal diet. Note persistence of nodularity. Histologic sections showed extensive areas of structurally normal tissue and a decrease in scarring.

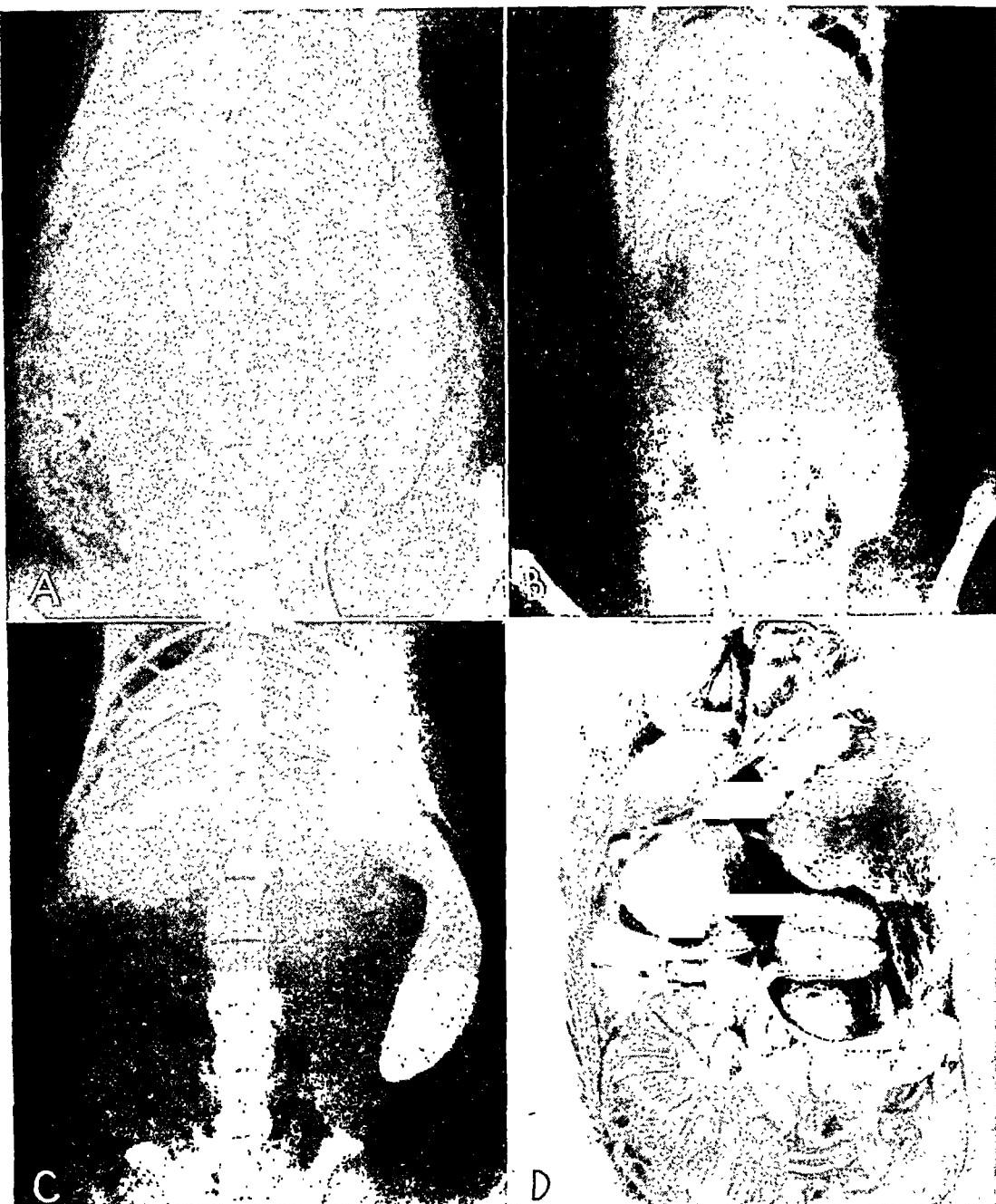


Fig. 4.—Recovery of a cirrhotic liver after five months of the normal diet. *A*, roentgenogram before feeding of butter yellow. *B*, roentgenogram after four months of the diet containing butter yellow. Note the configuration of moderate cirrhosis in small ring shadows. *C*, roentgenogram five months after return to the normal diet. Note the return to a diffuse shadow and the absence of ring shadows, indicating absorption of connective tissue.

*D*, gross photograph of the liver five months after return to the normal diet. Note absence of nodules and smooth surface, also one area of cancer.

After the fifth month the structure of the liver in some animals appeared normal. Little of the connective tissue which had subdivided the parenchyma remained. There were, however, multiple foci of cell degeneration, bile duct proliferation and new growth. Parts of livers had retained the nodularity and the connective tissue.

Increase in size of ring shadows on roentgenograms corresponded to reduction of or disappearance of connective tissue with presence of large areas of structurally normal liver. Thin walls of ring shadows indicated reduction in connective tissue. Thorium dioxide reappeared in small quantities in the sinusoidal capillaries. Absence of thorium dioxide shadows was associated with new growth. After the appearance

TABLE 2.—*Correlation of Pathologic Changes with Roentgenographic Appearances of Cirrhotic Livers After Return of Animals to a Normal Diet*

Rats	Duration of Butter Yellow-Rice- Carrot Diet, Months	Duration of Normal Diet	Roentgenographic Appearance	Pathologic Changes
2	4	2 wk.	No changes	No changes
4	4	6 wk.	Increase in size of shadow ring; decrease in ring walls	Decrease in connective tissue; larger structurally normal liver area
4	4-5	2 mo.	Progressive improvement up to 5th month, with reduction in number of ring shadows and appearance of patchy diffuse areas	Progressive improvement up to the 5th month, with reduction of scar tissue and return of structurally normal tissue
4	4-5	3 mo.	In some animals livers became normal; in others there was slight to marked improve- ment, with disappearance of ring shadows and return of diffuse areas of thinner than normal density	Return to normal in some livers; slight to marked im- provement in others; dis- appearance of most of scar tissue in some rats; degenera- tive, hyperplastic and new growth changes
2	4	4 mo.		
4	4	5 mo.		
2	4	6 mo.		
2	3	8½ mo.		

of new growth intracardial injections of the colloidal suspension of thorium dioxide failed to produce visualization. Apparently the thorium dioxide did not find its way into the blood vessels of new growths (fig. 4).

#### SUMMARY

Nodular cirrhosis of the liver was produced in albino rats by a diet of butter yellow, rice and carrot. A colloidal suspension of thorium dioxide injected intracardially visualized the liver of the normal animal as a diffuse roentgenographic shadow. As cirrhosis developed the roentgenographic shadow was altered. Ring shadows replaced the normal diffuse appearance. These ring shadows indicated the degree and the extent of the cirrhosis.

On return of animals to a normal stock diet, some of them showed progressive improvement, with partial or complete disappearance of connective tissue and nodular cirrhosis. The improvement was char-

acterized by gradual replacement of ring shadows by diffuse shadows. The gross and histologic changes were found to correspond with the roentgenographic alterations.

#### CONCLUSIONS

Connective tissue which is laid down in nodular cirrhosis induced by a butter yellow diet is reabsorbed in part or completely after the animals have been returned to a normal stock diet. There is also partial or complete restoration of the structural integrity of the hepatic parenchyma. The improvement becomes apparent within six weeks after the animal has returned to a normal diet. An approximate period of five months is required for significant absorption of connective tissue.

The progressive changes in nodular cirrhosis of the liver of the rat and the gradual return of the normal structure can be delineated roentgenographically by injecting intracardially a colloidal suspension of thorium dioxide.

The development of nodular cirrhosis and the return to normal are neither uniform in all animals nor uniform in the liver of the same animal.

# MICROSCOPIC LESIONS IN ACETYLCHOLINE SHOCK

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THE animal experiments reported here were undertaken for the purpose of ascertaining whether the administration of very large, and even lethal, doses of acetylcholine and neostigmine would produce definite microscopic changes of tissue. If such changes occurred, it should be decided whether they were of a nature to permit them to be classified as "shock," though, admittedly, microscopic changes in shock are not, as a rule, definitely characteristic.

From an analysis of its neurogenic effects, shock has been considered to be the result of an overstimulation of the sympathetic nervous system or, what is probably the same, an overaction of epinephrine. On the other hand, shock has also been regarded as due to hyperfunctioning of the parasympathetic system and thus as being possibly a sequel to strong parasympathicomimetic drug action.

The symptoms caused by strong doses of acetylcholine resemble so closely those in anaphylactic shock that "acetylcholine shock" has by some authors been considered to be closely related to anaphylactic shock if not actually identical with it (Nakamura,<sup>1</sup> Danielopolu<sup>2</sup>).

Danielopolu<sup>2</sup> suggested the name "paraphylactic shock" because of the absence of sensitization in acetylcholine shock. For a similar reason, the reaction to acetylcholine may be classified, according to Wells,<sup>3</sup> as anaphylactoid shock. (See also Moon<sup>4</sup> and Wiggers<sup>5</sup>.)

In recent years acetylcholine has been used to produce shock as a therapeutic agent in certain mental diseases, but the results so far have not been especially encouraging (Cohen, Thale and Tissenbaum;<sup>6</sup> Harris and Pacella<sup>7</sup>).

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From the Department of Anatomy, University of Saskatchewan.

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Most of the drugs used in the experiments reported in this paper were supplied by Mr. Paul Blanc (Hoffmann-LaRoche Ltd., Montreal, Canada).

1. Nakamura, K.: Jap. J. Exper. Med. **19**:31, 1941.

2. Danielopolu, D.: Deutsche med. Wochenschr. **69**:529, 1943.

3. Wells, H. G.: Physiol. Rev. **1**:44, 1923.

4. Moon, V. H.: Shock, Philadelphia, Lea & Febiger, 1942.

5. Wiggers, C.: Physiol. Rev. **22**:74, 1942.

(Footnotes continued on next page)

Farber, Pope and Landsteiner<sup>8</sup> observed that in only 10 per cent of the animals which had died from anaphylactic shock did the hearts show an increased acetylcholine content; in the other organs no increase was found.

Frommel, Thalheimer, Herschberg and Piquet<sup>9</sup> explained that the vagotonia in traumatic shock is caused by functional inhibition of cholinesterase action, allowing an increase of acetylcholine effect. The treatment of shock with cholinesterase (Schachter<sup>10</sup>) corresponds to this view.

In an earlier publication<sup>11</sup> one of us (R. A.) approached the problem of cholinergic shock in an experimental way, but the organs of the animals which had died after being injected with large doses of parasympathicomimetic drugs were not systematically examined.

#### MATERIALS AND METHODS

In the present study 24 white rats, 16 guinea pigs and 12 rabbits were shocked by intramuscular or intraperitoneal injections of strong lethal or sublethal doses of acetylcholine chloride, neostigmine methylsulfate or epinephrine hydrochloride. The doses were chosen and arranged so as to allow for varying intervals between the time of injection and the death of the animal. In several cases the doses were repeated at relatively short intervals, the animal dying after the last injection. The smallest single dose of acetylcholine chloride was 0.04 Gm. per thousand grams of body weight; the largest single doses, 0.42 Gm. per thousand grams of body weight. The smallest single dose of neostigmine methylsulfate was 0.2 cc. and the largest 3 cc. of a 1:2,000 solution per thousand grams of body weight. The shortest interval between injection and death was one minute; the longest between the first injection and death was one hundred and twenty-two minutes. Longer intervals (five hours) were noted with epinephrine. Moreover, numerous other animals were used for ascertaining threshold doses of the substances.

The lungs of the animals after death were fixed *in situ* by careful intratracheal instillation of Heidenhain's Susa fixative or of alcohol-solution of formaldehyde B.P. or solution of formaldehyde B.P. In a few instances the lungs were cut out after the trachea had been firmly ligated to prevent collapse of the lungs. But even in these specimens some injection fluid was gently instilled intratracheally so as to permit partial fixation from within.

The abdominal organs were first fixed *in situ*; after a few hours they were cut out, trimmed into blocks and refixed.

Various histologic stains were used: Hematoxylin-eosin, acid hematoxylin of Mallory, which stains fibrin well, and Mallory's connective tissue stain, which

6. Cohen, L. H.; Thale, T., and Tissenbaum, M. J.: Arch. Neurol. & Psychiat. **51**:171, 1944.
7. Harris, M. M., and Pacella, B. L.: Arch. Neurol. & Psychiat. **50**:304, 1943.
8. Farber, S.; Pope, A., and Landsteiner, E.: Arch. Path. **37**:275, 1944.
9. Frommel, E.; Thalheimer, M.; Herschberg, A. D., and Piquet, J.: Helvet. physiol. acta **1**:451, 1943.
10. Schachter, R. J.: Am. J. Physiol. **143**:552, 1945.
11. Altschul, R.: J. Nerv. & Ment. Dis. **99**:895, 1944.

shows the filling stage of blood vessels; further, iron and iron pigment stains and the Foot-Hortega reticulin stain were used.

The lungs, the heart, the liver, the kidneys, the spleen and the large and the small intestine were regularly searched. In a few cases the brain and the adrenal glands were also examined microscopically.

## RESULTS

The results may be grouped according to the animal species and according to the drug used (viz., acetylcholine, neostigmine or, for comparative reasons, epinephrine). No pathologic changes were observed in the intestines, and therefore these will not be described in the following report. Similarly, the findings in the brain and in the adrenal glands will be omitted, since these organs were not searched regularly.

*White Rats.*—(a) Acetylcholine and Neostigmine: The ventricles of the heart were found either in systole or in diastole, with no relation to the drug used, sometimes the left being in systole and the right in diastole. The myocardium showed capillary and venous hyperemia. In several specimens the arterioles were strongly contracted.

The kidneys revealed prevalently venous and capillary hyperemia, the liver prevalently venous and sinal hyperemia and the spleen moderate or no hyperemia.

In the majority of the rats there was capillary and venous hyperemia of the lungs. Intra-alveolar hemorrhages were observed in a few. Of 7 animals given intramuscular injections of acetylcholine chloride, 3 showed edema, 1 questionable edema and 3 none. No edema was found in another animal which had received acetylcholine chloride intraperitoneally. Of 5 animals shocked with intramuscular injections of neostigmine methylsulfate, 3 showed edema of the lungs and 2 did not. Of 5 rats treated with intraperitoneal injections of neostigmine methylsulfate, 1 showed a very slight degree of edema, while 4 showed no edema.

(b) Epinephrine: Seven rats were given injections of epinephrine hydrochloride for control purposes. Four which had received the drug intramuscularly showed intensive pulmonary edema, while of 3, in which the drug had been injected intraperitoneally, 1 showed no pulmonary edema and 2 showed very slight or doubtful edema of the lungs.

*Guinea Pigs.*—(a) Acetylcholine and Neostigmine: As in the rats, the ventricles of the heart were found in systole or in diastole, with no relation to the drug used. The veins and the capillaries were hyperemic in the majority of the animals, but the arterioles showed no definite contraction.

The kidneys generally showed venous hyperemia, mostly cortical. In half of the animals the arterioles were strongly contracted.

Various degrees of sinal and venous hyperemia were observed in the liver. The vasodilatation was more accentuated in those guinea pigs in which the drug had been injected intraperitoneally. In 3 animals the arterioles were extremely contracted. In 2, necrotic areas of the parenchyma were found, which probably were not connected with the state of shock, since the latter lasted in these animals eleven minutes and one hundred and six minutes, respectively. Two specimens appeared to be completely normal.

In the spleen all degrees of sinal and venous hyperemia were noted, but in some specimens the vessels appeared normal. In 1 animal, which had been given acetylcholine chloride intramuscularly and in 1 which had received neostigmine methylsulfate, also intramuscularly, the spleen appeared very "juicy" (edematous).

Of 8 animals given acetylcholine chloride intramuscularly, 3 showed pulmonary edema; 3 did not. In 1 the edema, if present, was of such little intensity that it may be classified as questionable. The lungs of 1 animal had to be discarded since they presented signs of incipient pneumonia, which obscured the investigation. Four animals received neostigmine; of 3 in which the drug was intramuscularly injected, 2 reacted with strong edema of the lungs, while the third showed only a very slight, questionable edematous reaction. Two animals in which the drug was intraperitoneally injected also showed very slight, even questionable pulmonary edema.

(b) Epinephrine: Epinephrine hydrochloride injected intramuscularly in 3 animals caused strong edema, but when injected intraperitoneally it caused in 1 animal a distinctly lesser pulmonary edema and no edema in 1 other.

*Rabbits.*—(a) Acetylcholine and Neostigmine: Systolic and/or diastolic standstill of the cardiac ventricles occurred without relation to the drug used, to the way of its administration or to the duration of the shock. There was moderate or no hyperemia of the myocardial capillaries. In the majority of the specimens venous hyperemia was noted; the arteries and the arterioles were only slightly contracted or not contracted at all.

The liver showed various degrees of sinal and venous hyperemia, ranging from strong to slight. In some specimens hyperemia was lacking. The arteries and the arterioles appeared to be normal.

In the majority of the rabbits the sinuses and the veins of the spleen were strongly hyperemic. In 2 animals the organ was distinctly edematous.

Acetylcholine chloride intramuscularly injected gave no edema in the lungs of 1 animal and slight edema in those of 1 other. Intraperitoneally applied in 1 animal, it caused no edema. Neostigmine methylsulfate, intramuscularly injected in 2 animals, did not provoke edema. Intraperitoneally injected it caused moderate edema in 2 animals but no edema in 2 others.

(b) Epinephrine: Epinephrine hydrochloride when given intramuscularly in 1 animal produced strong edema of the lungs, but when given intraperitoneally in another animal, provoked only moderate pulmonary edema.

#### COMMENT

Microscopic examination of various organs of animals which have been subjected to large doses of acetylcholine chloride or neostigmine methylsulfate shows great variation of the filling stage of blood vessels and frequent, but not regularly occurring, pulmonary and splenic edema. These changes correspond to the anatomic picture of shock.

As is well known, epinephrine also causes pulmonary edema. Thus we are confronted with the fact that antagonistic substances—two parasympathicomimetic and one sympathicomimetic drug—elicit the same change, i. e., pulmonary edema.

However, further study may reveal that there are different mechanisms producing edema in the cases of epinephrine-induced edema and of cholinergic edema.

The vascular changes and the pulmonary edema observed in white rats and guinea pigs were much more typical of shock than were the corresponding findings in rabbits. On the other hand, the epinephrine-

induced pulmonary edema in the rabbit was incomparably stronger than the cholinergic edema in the few cases in which this was produced. This is not surprising in view of the species-bound differences of cholinergic sensitivity (Altschul<sup>11</sup>) and also in view of the fact that, as Best and Taylor<sup>12</sup> have pointed out, the pulmonary vessels of the rabbit react with constriction to vagal stimulation, whereas in other animals vasodilatation occurs. But a review of this problem by de Burgh Daly<sup>13</sup> makes one doubt any final statements regarding the vegetative innervation of pulmonary vessels.

There are in the literature some other examples of apparently synergic action as well as of apparently paradoxical action of sympathicomimetic and parasympathicomimetic substances. Grollman<sup>14</sup> stated that "epinephrine exerts certain effects which indicate a parasympathetic action;" Danielopolu<sup>2</sup> ascribed to acetylcholine an amphotimetic action. Exophthalmos is usually considered to be due to sympathetic reaction, but Brunton<sup>15</sup> described exophthalmos produced in dogs with acetylcholine. In my own experience the exophthalmos produced in rats by rather strong doses of neostigmine surpasses even the exophthalmos induced with acetylcholine. It is possible that the mechanisms of the sympathogenic and the cholinergic exophthalmos are different, but the ultimate explanation of their nature has yet to be furnished.

Besides this, attention may be directed to the observations that acetylcholine and epinephrine exert identical action on the nictitating membrane (Morrison and Acheson<sup>16</sup>), that the sympathetic cerebral centers are excited by acetylcholine after removal of the frontal lobes (Stavraky<sup>17</sup>) and that epinephrine and neostigmine show mutual reenforcement in the guinea pig (Altschul<sup>11</sup>). Luco<sup>18</sup> found that "adrenaline increases the responses of denervated muscles to acetylcholine."

Thus the occurrence of either an acetylcholine shock or an epinephrine shock does not necessarily exclude the occurrence of the other; pulmonary edema caused by parasympathicomimetic substances will have to be accepted as well as that brought about by sympathicomimetic substances.

It is conceivable that the apparently paradoxical actions of acetylcholine and neostigmine are due to cholinergic mobilization of a large

12. Best, C. C., and Taylor, N. B.: *The Physiological Basis of Medical Practice*, ed. 3, Baltimore, Williams & Wilkins Company, 1943.

13. de Burgh Daly, I.: *Physiol. Rev.* **13**:149, 1933.

14. Grollman, A.: *The Adrenals*, Baltimore, Williams & Wilkins Company, 1936.

15. Brunton, C. E.: *J. Physiol.* **97**:383, 1940.

16. Morrison, R. S., and Acheson, G. H.: *Am. J. Physiol.* **121**:149, 1938.

17. Stavraky, G. W.: *Tr. Roy. Soc. Canada (Sect. 5)* **37**:127, 1934.

18. Luco, J. V.: *Am. J. Physiol.* **125**:196, 1939.

amount of epinephrine in the animal. A new series of experiments with scope surpassing that of the present work will have to be carried out on adrenalectomized animals in order to clarify the question of the ultimate mechanism in cholinergic shock and also of the other paradoxical phenomena of cholinergic action, referred to in the foregoing paragraphs.

The fact that acetylcholine and neostigmine, as well as epinephrine, acted much more strongly when administered intramuscularly than when given intraperitoneally deserves attention and perhaps further investigation. The slowing down of the action of epinephrine and of that of parasympathicomimetic drugs, as well, and the consequent increase of their threshold may be caused by different mechanisms. A tentative explanation is that in the first case the delay may be due to constriction of the peritoneal vessels, while in the second case it may be due to a diluting and delaying action by the blood reserve in the liver. Other factors may play minor or major roles.

#### SUMMARY

Sublethal and lethal doses of acetylcholine chloride, neostigmine methylsulfate and, for control purposes, epinephrine hydrochloride were injected into white rats, guinea pigs and rabbits.

Microscopic examination of various organs of the test animals showed a vascular reaction in many of them which resembled that described in other types of shock.

Moreover, in about half of the rats and the guinea pigs, the cholinergic drugs caused pulmonary edema. This supports still more the view that cholinergic shock may be accepted and correlated with other shock reactions.

The findings in the rabbits were less convincing, especially the reactions of the pulmonary vessels. This fact is not surprising since the pulmonary vessels of the rabbit give a constrictor response to vagal stimulation.

Attention is drawn to the fact that both epinephrine and cholinergic substances reacted much more slowly and weakly when they were applied intraperitoneally than when they were given intramuscularly.

The sympathetic and the parasympathetic stimulation produced several synergic and even paradoxical effects.

## RELATION OF CHOLELITHIASIS TO ACUTE HEMORRHAGIC PANCREATITIS

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**I**N 1901 Opie<sup>1</sup> reported a case of acute pancreatitis associated with a gallstone impacted in the ampulla of Vater. He suggested that a gallstone may produce pancreatitis in this way by diverting bile into the pancreatic duct. Cases of the type described by Opie are seldom seen at necropsies, but many pathologists have the impression that there is a high incidence of disease of the gallbladder in association with acute pancreatitis. However, no statistical evidence has been presented. Rich and Duff<sup>2</sup> maintained, on the contrary, that disease of the gallbladder is not unusually frequent in patients with acute pancreatitis; they attributed the pancreatic lesion to obstruction of the intrapancreatic ducts as a result of hyperplasia of the epithelial lining of these ducts.

The autopsy records of the department of pathology of the University of Minnesota show acute hemorrhagic pancreatitis encountered in 158 of 41,333 persons (26,262 males, 15,401 females) examined post mortem. Ninety-three of the patients were males, and 67 were females, but since there were about twice as many males as females among persons over 30 years of age coming to necropsy, the true proportion is about 10 males to 17 females.

TABLE 1.—*The Incidence of Cholelithiasis in Association with Acute Pancreatitis*

Decade of Life	Persons Showing Acute Pancreatitis		Persons Showing Acute Pancreatitis Associated with Cholelithiasis		Persons Showing Cholelithiasis at Necropsy According to Ludlow's Report	
	Males	Females	Males, %	Females, %	Males, %	Females, %
1.....	3	3	0	0	0.0	0.0
2.....	0	2	0	0	0.88	1.54
3.....	2	1	0	0	2.52	4.92
4.....	14	8	28.6	37.5	2.40	7.39
5.....	12	20	25.0	85.0	6.30	16.66
6.....	25	14	32.0	85.7	10.66	22.62
7.....	22	10	50.0	90.0	15.09	24.76
8.....	12	7	50.0	57.1	13.73	26.83
9.....	3	2	33.3	50.0	0.0	50.0
Total.....	93	67	36.3	68.7	5.77	10.45

In the accompanying table the frequency of cholelithiasis associated with acute pancreatitis in the aforementioned necropsy series is shown

From the Department of Pathology, University of Minnesota.

1. Opie, E. L.: Bull. Johns Hopkins Hosp. **12**:182, 1901.

2. Rich, A. R., and Duff, L. G.: Bull. Johns Hopkins Hosp. **58**:212, 1936.

along with Ludlow's<sup>3</sup> report of the occurrence of cholelithiasis in 4,800 persons (2,952 males, 1,848 females) examined post mortem. In each decade of life the number of cases of pancreatitis is too small to give a significant percentage, but the total group seems sufficiently large to justify the conclusion that cholelithiasis is observed much oftener in necropsies on persons with acute pancreatitis than in autopsies on people in general. It is clear that in about two thirds of the males and one third of the females with acute pancreatitis this disease develops in the absence of gallstones, but since gallstones are found about six times as frequently in both males and females with acute pancreatitis as in the necropsy population in general there must be some causal relation between the two findings. The mechanism by which gallstones produce pancreatitis has not been fully explained, but there is some evidence for the older view that they produce temporary obstruction of the papilla of Vater and divert bile into the pancreatic ducts.

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3. Ludlow, A. I.: Am. J. M. Sc. **193**:481, 1937.

# EXPERIMENTAL PITUITARY DIABETES OF FIVE YEARS' DURATION WITH GLOMERULOSCLEROSIS

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AND

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PHILADELPHIA

FOLLOWING the discovery of insulin and the finding that raw pancreas, as well as insulin, was required for the extended survival of depancreatized dogs, several investigators observed diabetic dogs for long periods. As no comparable reports on pituitary diabetes have appeared, this account of a dog with that type of diabetes, which was studied for five years, is presented. In table 1 the literature on experimental diabetes of long duration has been collected. According to this table, the case presented in the following pages is the fourth case of experimental diabetes and the first of pituitary diabetes of five years' duration to be recorded.

The diabetes produced in this dog with a crude saline extract of carefully dissected anterior lobes of beef pituitary glands<sup>1</sup> and some of the metabolic studies have been described in previous reports. The animal was first listed as dog I<sup>2a</sup> and later as dog P 16.<sup>2b,c</sup> Metabolic studies subsequent to those reports have shown no important changes, so that this type of data need not be repeated. The persistence and the constant degree of severity of the diabetes are shown in table 2, which requires brief comment. The period of the injection of the pituitary extract has been excluded, and data obtained during any kind of treatment have been omitted from table 2. The blood chemical values are averages of five to twenty determinations except in the instance of the serum lipids, which were measured only twice in the fifth year. The proportion of the available carbohydrate of the diet excreted in the urine is used as an approximate measure of the severity of the disease. (It is calculated from a dextrose-nitrogen

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From the George S. Cox Medical Research Institute, University of Pennsylvania.

1. The preparation of the extract has been described elsewhere (Young, F. G.: Biochem. J. **32**:513, 1938).

2. (a) Dohan, F. C., and Lukens, F. D. W.: Am. J. Physiol. **125**:188, 1939. (b) Dohan, F. C.; Fish, C. A., and Lukens, F. D. W.: Endocrinology **28**:341 (March) 1941. (c) Dohan, F. C.; Chambers, A. H., and Fish, C. A.: ibid. **28**:566, 1941.

TABLE 1.—*Summarized Review of Literature on Prolonged Experimental Diabetes*

Reference	Species (Animals)	Duration of Diabetes	Lesions
Diabetes Produced by Total Pancreatectomy			
Hedon, E.: Compt. rend. Soc. de biol. <b>100</b> : 698, 1929	Dog (1)	5 years	Renal damage
Macleod, J. J. R.: Carbohydrate Metabolism and Insulin, London, Longmans Green & Co., 1926, p. 88	Dog (2)	4 years	Fatty liver, aortic lesions
Chaikoff, I. L., and Kaplan, A.: J. Nutrition <b>14</b> : 459, 1937	Dog (4)	2 for 4 years 2 for 5 years	Cataracts, lipemia, fatty liver
Dragstedt, L. R.; Goodpasture, W. C.; Vermeulen, C., and Clark, D. E.: Am. J. Physiol. <b>126</b> : 479, 1939	Dog (6)	Not stated	Arteriosclerosis
Diabetes Produced by Partial Pancreatectomy			
Sandmeyer, W.: Ztschr. f. Biol. <b>31</b> : 12, 1895	Dog (2)	2 and 8 months	Cachexia only
Langfelt <sup>3</sup> .....	Dog (4)	9, 12, 18 and 20 months	Hyperplasia of thyroid gland
Fisher, N. F.: Am. J. Physiol. <b>67</b> : 634, 1923	Dog (1)	8 months	Arteriosclerosis
Diabetes Produced by Injections of Pituitary Extract			
Richardson, K. O., and Young, F. G.: Lancet <b>1</b> : 1098, 1938. Richardson <sup>4</sup>	Dog (5)	25 to 58 weeks	Pancreatic lesions only
Ham, A. W., and Haist, R. E.: Am. J. Path. <b>17</b> : 787, 1941	Dog (3)	78 to 198 days	Pancreatic lesions only
Dohan and others <sup>2b</sup> .....	Dog (6)	92 to 418 days	Pancreatic lesions only
This report .....	Dog (1)	5 years	Pancreatic and renal lesions
Lukens, F. D. W., and Dohan, F. C.: Endocrinology <b>30</b> : 175, 1942. Lukens, F. D. W.; Dohan, F. C., and Wolcott, M. W.: Ibid. <b>32</b> : 475, 1943	Cat (14)	142 to 463 days	Pancreatic lesions only
Diabetes Produced with Alloxan			
Bailey, C. C.; Bailey, O. T., and Leech, R. S.: New England J. Med. <b>230</b> : 533, 1944	Rabbit	Late findings	Pancreatic and renal lesions of alloxan
Goldner, M. G., and Gomori, G.: Proc. Am. Diabetes A. <b>4</b> : 89, 1944	Dog, rat	Unreported	Cataract

TABLE 2.—*Course of Diabetes of Dog P 15*

Year of Diabetes	Serum						Time Treated per Year, Days
	Blood Sugar, Mg. per 100 Cc. (Average)	Fatty Acids, per Cent (Average)	Cholesterol, Mg. per 100 Cc. (Average)	Glycosuria, per Cent of Dietary Carbohydrate	Ketonuria		
1	200	0.641	...	76	0 to 4+	53	
2	198	0.552	365	87	0 to 4+	91	
3	219	0.513	396	82	0 to 1+	153	
4	194	.....	...	86	0 to 2+	0	
5	217	0.860	210	84	0 to 1+	46	

ratio of 3.65.) The average value for glycosuria is based on two week metabolic periods near the end of each year. In the period for the first year the diet was 1,000 Gm. of beef daily. In the other metabolic

periods it was 700 to 800 Gm. per day. The practically uniform diet during the later periods adds validity to this index of the constancy of the diabetes.

#### TREATMENT

The treatment of this animal included injections of insulin, reduction of the diet and one course of treatment with phlorhizin. In the first year the treatment consisted of numerous short periods of dietary restriction. The first course of insulin treatment was not begun until the five hundred and thirty-seventh day of diabetes (four hundred and ninety-four days after the last injection of the pituitary extract). Insulin was used for fifty-three days, and fairly good control of the diabetes was achieved, the morning blood sugar ranging from 63 to 177 mg. per hundred cubic centimeters during treatment. Glycosuria was reduced from an average of 61 Gm. to about 5 Gm. per day on the same diet. Short courses of insulin therapy were given in the third and fifth years, but insulin was used for a total of only one hundred and eighty-six days of the animal's five years of diabetes. The insulin requirement from year to year is shown in table 3:

TABLE 3.—*Insulin Requirement of Dog P 16*

Year	Meat, Gm. per Day	Insulin, Units per Day
2d.....	800	32
3d.....	700	30
5th.....	800	36

The failure to lessen the diabetes by treatment begun late in the disease (i. e., four to six weeks after the onset of glycosuria in dogs) has been described before.<sup>2b</sup> There was one thirty-four day period of a pure fat diet, during which the animal showed only traces of glycosuria, ketonuria (4 plus) and a blood sugar level of 169 to 175 mg. per hundred cubic centimeters. Otherwise, periods of dietary treatment were never longer than three to six day metabolic periods. They have been included to indicate the time during which the diabetes was temporarily altered by changes in regimen. It is clear that the total time of all types of treatment was a small fraction of the five years of diabetes, and that these types of treatment did not influence the course of the disease. The animal's weight was maintained during the long periods of maintenance diet without treatment.

For the two weeks preceding its death the dog was in excellent condition on a diet of 750 Gm. of meat daily and excreted an average of 68 Gm. of dextrose per day. Traces of acetone were present in the urine. The animal weighed 8.5 Kg. at the beginning of the experiment and 11.6 Kg. at autopsy five years later. Autopsy was

begun with the animal under pentobarbital sodium anesthesia in order that glycogen might be determined.

#### TERMINAL CHEMICAL DATA

Hepatic glycogen was evaluated as 1.14, cardiac glycogen as 0.4 and renal glycogen as 0.23 per cent. The blood sugar amounted to 242 mg., the blood urea nitrogen to 21 mg. and the serum cholesterol to 208 mg. per hundred cubic centimeters. The values for fatty acids were, serum 0.90, liver 22.0, and diaphragm 0.33, per cent.

#### NECROPSY

The pancreas weighed 30 Gm. and was grossly normal. The liver weighed 600 Gm. and was very fatty and friable. The two adrenal glands together weighed 1.56 Gm. and appeared normal. The kidneys were pale with yellowish striations. Other organs were grossly normal.

#### MICROSCOPIC EXAMINATION

No abnormalities were found in the following organs: heart, lungs, stomach, duodenum, spleen, adrenal glands, thyroid gland and parathyroid glands. The normal thyroid gland, with flat or cuboidal epithelium lining the acini, is noted in contrast to the gland showing hyperplasia reported by Langfelt.<sup>3</sup> The splenic and coronary blood vessels were unchanged. The eyes were found normal by Dr. Irving Leopold, of the department of ophthalmology of the University of Pennsylvania.

In the pancreas the acinous tissue and the blood vessels were normal. There was slight vacuolation of the epithelium of the small ducts.<sup>4</sup> The islands of Langerhans were slightly fewer than normal, and all were small and irregular in shape (fig. 1). Bensley stains were not made, but even with hematoxylin and eosin it seemed that some beta cells were present. There was hydropic degeneration in an occasional beta cell. The Masson and Mallory stains revealed no hyaline change in the islands. The condition of the islands conformed to previous descriptions of the atrophy seen late in pituitary diabetes.<sup>5</sup>

The hepatic parenchyma was extensively and diffusely infiltrated with fat, and this was confirmed by fat staining. The ducts and the vessels were normal, and there was no cellular or inflammatory reaction and no excess of connective tissue.

The kidneys showed glomerular and tubular lesions. The glomerular lesions resembled the early changes of intercapillary glomerulosclerosis as described and reviewed by Allen.<sup>6</sup> All of the glomeruli

3. Langfelt, E.: Acta med. Scandinav. **53**:1, 1920.

4. Richardson, K. C.: Proc. Roy. Soc., London, s.B **128**:153, 1940.

5. Richardson.<sup>4</sup> Dohan and others.<sup>2b</sup>

6. Allen, A. C.: Arch. Path. **32**:33, 1941.

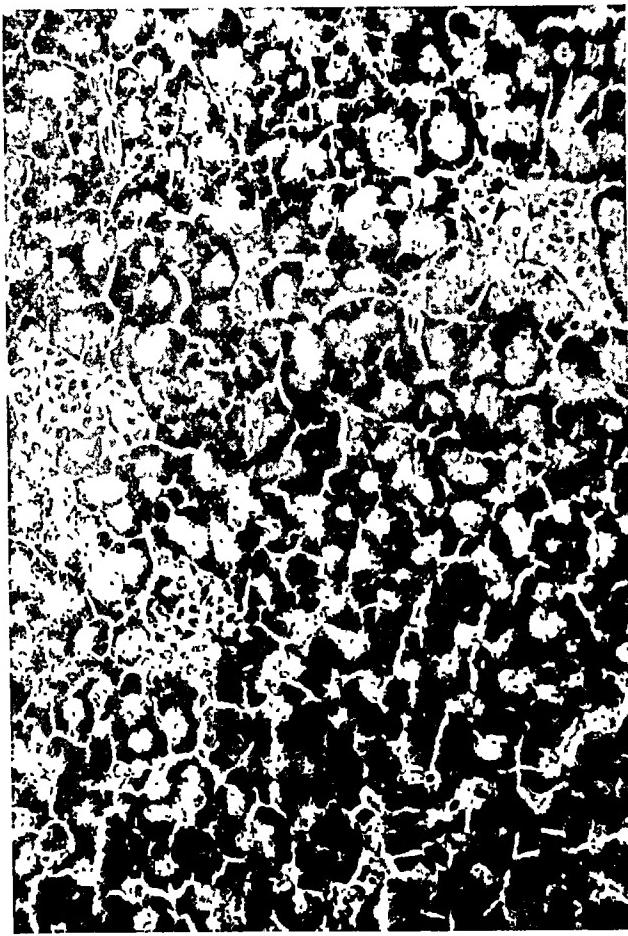


Fig. 1.—Pancreas;  $\times 177$ .

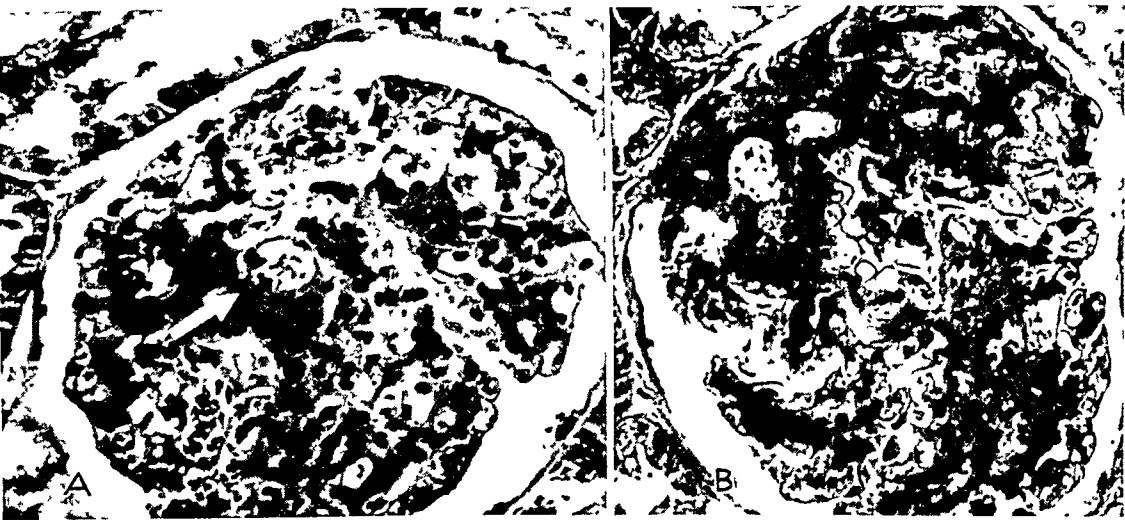


Fig. 2.—In A the thickened capillary wall surrounding a lumen containing erythrocytes is shown by the arrow. Another such capillary is seen beyond it in the direction of the arrow. Focal cellular proliferation of the type shown here was present in all glomeruli. Hematoxylin and eosin; high power magnification. In B the patchy deposition of hyalin in a glomerulus is shown by the Masson stain under high power magnification.

had small focal hyaline deposits in the walls of the capillaries. The hyalin took the form of a thin ring or crescent of eosinophilic material next to the lumen and was also seen as irregular deposits (fig. 2). In all glomeruli the hyaline change was overshadowed by the striking focal cellular proliferation. The cells, which appeared to be endothelial, were most conspicuous around the afferent arterioles. Occasional arterioles outside the glomeruli were also involved. There were no leukocytes or other evidence of inflammation. In his review Allen<sup>6</sup> noted that the cellularity of the lesions of glomerulosclerosis varied widely, and his illustrations suggest that the cellular collections are more prominent when the hyaline change is minimal or early. Hyaline thickening of Bowman's capsule was frequently observed.

The tubules were the site of extreme patchy fatty infiltration, which fat staining showed was accompanied by fine fatty deposition throughout the organ, including the glomeruli. Such fatty changes have been described in the kidneys of diabetic and some nondiabetic patients. The larger vessels and collecting tubules were not remarkable.

#### COMMENT

The observation that pituitary diabetes was present for five years after the cessation of injections of pituitary extract supports the use of the term "permanent" in describing this diabetes. After the first year of the condition, neither hyperglycemia nor any other factor increased the severity of the disease. The constancy of this dog's essentially untreated diabetes resembled that observed in certain cases of human diabetes. At autopsy the lesions were limited to those of diabetes. The atrophy of the islands of Langerhans and the fatty deposits in the liver and the kidneys conform to previous descriptions. The unexpected occurrence of "intercapillary glomerulosclerosis" provides the first example known to us of this lesion in an animal. It is generally regarded as a diabetic lesion, although this statement is not above controversy. The glomerular and vascular lesions in this dog were distinct from the lymphocytic infiltration and scarring of chronic interstitial nephritis which occurs spontaneously in dogs.<sup>7</sup>

#### SUMMARY

Diabetes produced in a dog by injections of a pituitary extract was observed for five years and found to be of constant severity after the first year. At autopsy the lesions were those which have been previously noted in cases of diabetes, viz., fatty deposits in the liver and the kidneys. In addition, intercapillary glomerulosclerosis, which has not hitherto been reported in experimental diabetes, was noted.

7. Morehead, R. P., and Little, J. M.: Am. J. Path. 21:339, 1945.

## MORPHOLOGIC STUDIES OF RATS DEPRIVED OF ESSENTIAL AMINO ACIDS: III. HISTIDINE

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AND  
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DETROIT

YOUNG rats fed synthetic diets devoid of a single essential amino acid survived an experimental period of one month with difficulty; during this period the rats lost weight, became very weak and lost their healthy appearance.<sup>1</sup> Examination of rats after they had consumed diets in which either phenylalanine or leucine was absent revealed marked thymic atrophy, atrophy and decrease of lipids of the adrenal cortex, degeneration and atrophy of the epithelium of the seminiferous tubules and thinning of the epiphyses of the long bones. In addition, leucine-deficient animals had striking ocular changes, similar to those previously reported by investigators<sup>2</sup> who studied tryptophane and lysine deficiencies. These changes consisted of thinning and metaplasia of the corneal epithelium with edema and leukocytic infiltration of the substantia propria.

In observations reported thus far by us reductions of hemoglobin and plasma protein have been noted in animals on diets deficient in phenylalanine<sup>1a</sup> or lysine,<sup>3</sup> but alterations of this type were not found in rats fed leucine-deficient rations.<sup>1b</sup> The observations previously reported by us were made in acute experiments during which the animals were entirely deprived of a single essential amino acid so that their food consumption was markedly diminished and the animals rapidly lost weight. The anatomic alterations might be considered to be due in part at least to general malnutrition.

Histidine, the third amino acid to be studied, represents a heterocyclic type of amino acid. The two substances previously investigated,

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1. Maun, M. E.; Cahill, W. M., and Davis, R. M.: (a) Arch. Path. **39**:294, 1945; (b) **40**:173, 1945.

2. Buschke, W.: Arch. Ophth. **30**:735, 1943.

3. Allen, E.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 22.

phenylalanine<sup>1a</sup> and leucine,<sup>1b</sup> represent the aromatic and aliphatic types of amino acids, respectively. Although histidine is required for normal growth, it is synthesized in the adult rat and in man as evidenced by the fact that nitrogen balance can be maintained when this amino acid is missing from the diet.<sup>4</sup> Since histidine may be dispensable in the nutrition of adult rats, one might expect less manifest alterations in the health and the tissues of young animals restricted to diets completely devoid of this amino acid.

#### EXPERIMENTAL PROCEDURE

*Animals.*—Weanling rats of the Fisher line, 344 strain, which were 30 days old, were pair-fed throughout the experiment in individual cages. All of the eleven pairs of rats survived the twenty-eight day experimental period and were then killed by decapitation without anesthesia.

*Diets.*—The rats were fed purified diets<sup>1a</sup> of crystalline amino acids, crystalline vitamins, fats and sucrose plus the necessary salts. The adequate control diets contained 6.4 Gm. of histidine per kilogram, which was replaced in the deficient diets by 6.4 Gm. of sucrose. The average food consumption per rat in both groups was 2.98 Gm. per day.

*Autopsies.*—Examinations were made immediately after the animals were killed, each organ being routinely inspected and weighed. The entire organ or a portion thereof was fixed in Zenker's fluid, and one each of the paired organs with the exception of the eyes was fixed in Bouin's solution. Additional sections were fixed in a 4 per cent solution of formaldehyde. The tissues other than the eyes were embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. Other stains were employed in particular instances as necessity demanded.

#### RESULTS

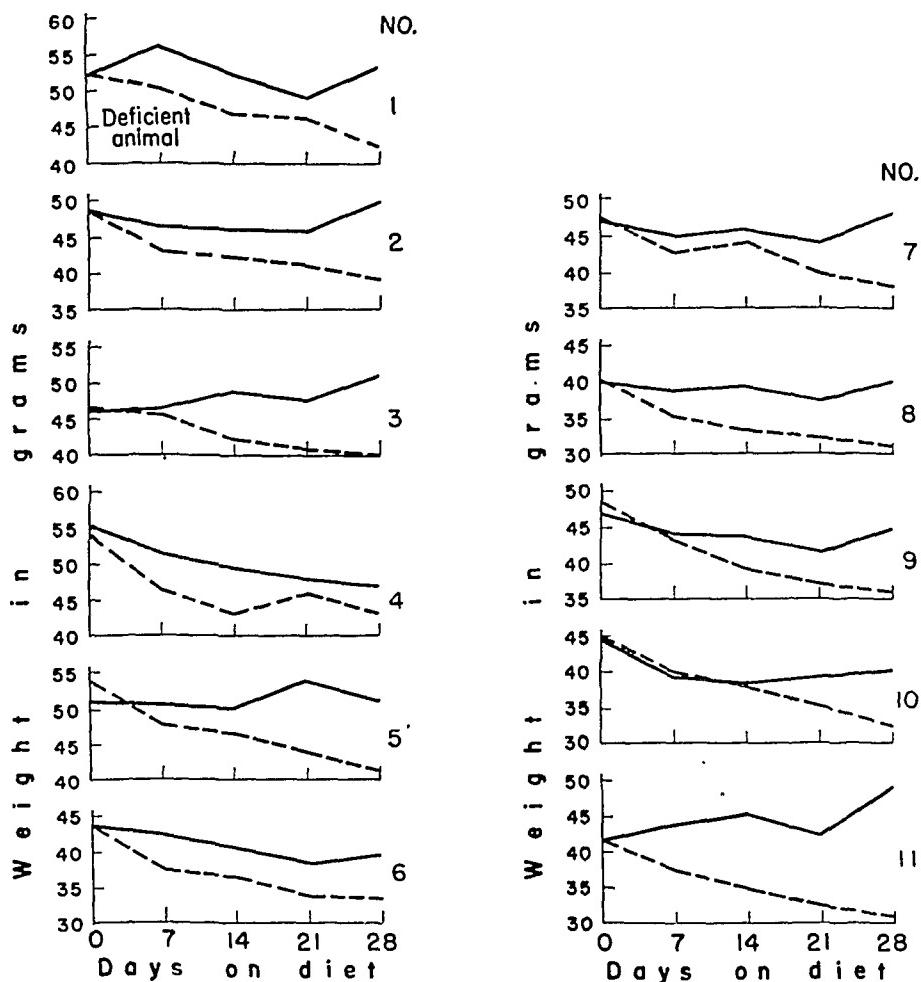
*Health.*—Animals consuming the deficient diets showed more lassitude than the control rats; the former appeared to sleep most of the time, and it was necessary to stimulate them to activity. The deficient animals showed no signs of hunger when fed, and near the close of the experimental period their coats appeared shaggy. The observed alterations were, however, much less pronounced than those that had been previously noted in the experiments in which the rats were fed phenylalanine-deficient or leucine-deficient diets.

*Changes in Weight.*—The animals fed deficient diets showed an average loss of weight of 10.2 Gm. each, whereas the control animals lost an average of 0.7 Gm. each. It is noteworthy that every rat on a histidine-free diet lost weight, while some of the control animals failed to lose weight although the caloric value of their diets equaled that of the experimental diets (figure).

4. Rose, W. C.; Haines, W. J.; Johnson, J. E., and Warner, D. T.: J. Biol. Chem. **148**:457, 1943. Burroughs, E. W.; Burroughs, H. S., and Mitchell, H. H.: J. Nutrition **19**:363, 1940.

*Organ Weights.*—Each organ was weighed immediately after its removal, and the percentage of the organ weight was determined. (The percentage of an organ weight =  $\frac{\text{organ weight}}{\text{body weight}}$ ). The weights were compared in each experiment, and the totals were submitted to statistical analysis. The only consistent variation was noted in the thymuses.

*Roentgen Studies.*—Several days prior to the end of the experimental period, roentgen studies were made of the complete skeletons of both the



The more rapid loss of weight of histidine-deficient rats as compared with their pair-fed controls. The broken line represents the deficient rat in each pair.

control and the deficient animals. There were no differences in the two groups.

*Hemoglobin.*—Determinations of the hemoglobin content of the blood were made by the Sheard-Sanford method<sup>5</sup>; the hemoglobin was found

5. Sheard, C., and Sanford, A. H.: J. Lab. & Clin. Med. **14**:558, 1929.

to be consistently lower in the deficient animals, which averaged 10.4 Gm. per hundred cubic centimeters, whereas the control animals averaged 14.1 Gm. pr hundred cubic centimeters.

*Plasma Proteins.*—Total plasma proteins were determined by the micro-Kjeldahl method with the aid of a Pregl<sup>6</sup> distillation apparatus. Only slight alterations were found, the average for the histidine-deficient rats being 5.42 Gm. per hundred cubic centimeters, compared with 6.19 Gm. for the control animals.

*Hepatic Fats.*—The fats of the liver were determined by the method of Leathes and Raper,<sup>7</sup> and the two groups of animals were found to have similar amounts per hundred grams of liver.

#### MORPHOLOGIC OBSERVATIONS

*Thymus.*—On gross inspection the thymuses of the deficient rats were notably smaller than those of the controls, the weight of the control glands being  $48.9 \pm 11.9$  mg., compared with the weight of the deficient glands  $14.3 \pm 2.3$  mg. The atrophy, on gross inspection and on histologic examination, was much less marked than that noted in either phenylalanine-deficient or leucine-deficient animals, but the pattern of the observed atrophy was similar.

The structure of the histidine-deficient glands was well preserved, and the cortical and medullary zones were distinct; however, an obvious thinning of the lymphoid elements of the cortex made the stromal portions of the glands more prominent. Inspection under higher magnification disclosed fibroblastic proliferation, and a few nests of macrophages were seen in the medulla. Giant cells were not observed. The thymic glands of the deficient animals were of sufficient size to permit the preparation of frozen sections, which were stained for lipids. When these were compared with the control material, many tiny droplets of lipids, stainable with Sudan III but not with nile blue, were seen to be scattered throughout the gland but were more prominent in the cortex. These lipids appeared to be within the cytoplasm of the reticular cells.

*Adrenal Glands.*—The glands from both groups of animals were comparable in weight. Examination of the prepared sections stained with hematoxylin and eosin showed little evidence of compression of the middle zone or cellular atrophy such as that previously noted in the

6. Pregl, F.: Die quantitative organische Mikroanalyse, ed. 2, Berlin, Julius Springer, 1912.

7. Leathes, J. B., and Raper, H. S.: The Fats, ed. 2, New York, Longmans, Green & Co., 1925.

phenylalanine-deficient and leucine-deficient rats. Frozen sections of these glands were stained with Sudan III, and on examination the adrenal glands of the histidine-deficient rats showed a slight decrease of the lipids in the zona glomerulosa and in a few patchy areas in the outer portion of the middle zone, but the lipids stainable with Sudan III proved to be almost equal to those seen in the adrenal glands of the control animals.

*Hypophysis.*—The hypophyses from the control and the histidine-deficient rats showed no significant differences in weight. After the glands had been fixed in Zenker's solution, sections were made and stained with hematoxylin and eosin, Mallory's trichrome stain and azocarmine. On examination of the sections, no cellular changes were found, and the distribution of cells was similar in the deficient and the control animals.

*Bone and Marrow.*—Examination of sections of bone taken from the sternum and the vertebrae and of longitudinal sections of long bones showed alterations similar to, but less pronounced than, those previously described in animals maintained on phenylalanine-deficient and leucine-deficient diets. The epiphyses were narrowed, and the trabeculae were blunt and short. The degree of calcification was equal to that found in control animals, although the calcified areas were observed to stain irregularly. Detailed studies of the marrow were not possible because of unsatisfactory preservation of the marrow elements; however, there appeared to be no pronounced alterations between those of the control and those of the deficient group.

*Male Genital System.*—On gross inspection the testes of the two groups of animals were comparable in appearance and were found to be about equal in weight. Examination of the prepared sections revealed that the spermatogenesis of the histidine-deficient animals was delayed as compared with that of the control animals but was more advanced than that noted in rats fed phenylalanine-deficient and leucine-deficient diets. Spermatogonia and primary spermatocytes could be readily distinguished, but degenerated cells often occluded the lumens. Mitoses were abundant in the control animals, and in a few instances spermatids were seen, but mature spermatozoa were not noted in any of the deficient animals. The interstitial cells were unaltered in both groups. Examination of the prostates and seminal vesicles showed them to be comparable in the histidine-deficient and the control animals.

*Eyes.*—During the experiment, the eyes of the animals were repeatedly inspected, but no distinct gross differences were found. After the

eyes had been fixed in Zenker's fluid, they were embedded in celloidin and cut at 5 microns. The sections were stained with hematoxylin and eosin and with Mallory's trichrome stain. The eyes from the control animals were essentially normal.

The changes previously noted in leucine-deficient rats were also present but were less striking in rats on histidine-deficient diets. These consisted of thinning and stratification of the corneal epithelium with keratin formation on the surface. The substantia propria appeared thickened, but the structural pattern was well preserved. The vessels immediately beneath the corneal epithelium were dilated and prominent, and in a few instances scattered leukocytes were present in the perivascular tissue. Descemet's membrane was more prominent than in the control animals, but the anterior chambers were free of exudates. The structure of the remainder of the eyes was normal.

*Other Organs and Tissues.*—The remaining organs were inspected and, when it was feasible, weighed and sections of each were prepared for examination. All sections were stained routinely with hematoxylin and eosin. Best's carmine stains were used for the liver specimens and cresyl violet was employed to stain tissues of the central nervous system. The following tissues and organs of the histidine-deficient rats, when compared with the control animals, were found to present no significant alterations: heart, lungs, liver, spleen, pancreas, kidneys, voluntary muscle, skin, urinary bladder, salivary glands, thyroid gland, parathyroid glands, female reproductive system and the central and peripheral nervous system.

#### COMMENT

Animals fed histidine-deficient diets lost more weight than did their pair-fed partners, but the deficient rats appeared healthier than rats maintained on either phenylalanine-deficient or leucine-deficient diets. It is noteworthy that rats on the diets free of histidine had low hemoglobin (averaging 10.4 Gm. per hundred cubic centimeters of blood, which is comparable with the hemoglobin value found in phenylalanine deficiency). As might be predicated, since the growing rat is able to synthesize some of its required histidine, the anatomic alterations were similar to, but less evident than, those observed in other amino acid deficiencies. The experiment recorded here may have been too brief to permit the development of extensive anatomic alterations.

#### SUMMARY

Young inbred rats were fed purified diets made of crystalline amino acids, crystalline vitamins, dextrin, hydrogenated cottonseed oil (crisco) and the necessary salts. The animals were pair-fed so that the food con-

sumption of the control animals was equal to that of the deficient animals whose diet was devoid of histidine. The deficient animals lost weight more rapidly throughout the experiment and were less active than the control animals. At the end of a twenty-eight day experimental period the histidine-deficient group showed decrease in hemoglobin, atrophy of the thymus and vascularization and epithelial metaplasia of the cornea.

# OCCURRENCE OF RHEUMATIC CARDITIS IN THE NATIVE POPULATION OF CURAÇAO, NETHERLANDS WEST INDIES

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It seems to be a widely accepted belief, at least in the American medical literature, that acute rheumatic fever and rheumatic carditis are extremely rare or do not occur in the tropics. According to MacCallum,<sup>1</sup> the disease is hardly to be found in the tropics. Boyd,<sup>2</sup> discussing the etiologic factors of acute rheumatic fever, cited Coburn, who pointed out that the incidence of acute rheumatic fever parallels the incidence of streptococcic diseases, such as scarlet fever, and that both are almost unknown in the tropics. A child suffering from recurrent attacks of rheumatic fever and streptococcic sore throat in the slums of New York should remain well when transported to South America. Forbus,<sup>3</sup> also citing Coburn, observed that "it has been known for a long time that acute rheumatic fever is a disease of temperate climates and is almost unknown in tropical regions except in areas of high altitude where the climate is severe." Steinbröcker<sup>4</sup> advised removal of patients with rheumatic carditis to the southern states and Puerto Rico. According to Hegler,<sup>5</sup> acute rheumatic fever is rare in the tropics. It is almost completely absent in the Antilles and Puerto Rico but more frequent in other tropical countries, e. g., India. Strong<sup>6</sup> stated that from a study of the statistical reports and from the writings of

various authorities there would seem to be two cosmopolitan diseases which are of extreme rarity in natives in the tropics: rheumatic fever and scarlet fever. He further pointed out that although 614 cases of rheumatic fever with one death were reported from the Gold Coast in 1911, there was no increase in admissions for vascular disease as would naturally be expected. Strong cited Manson-Bahr, who stated that there are some who never observed rheumatic fever or endocarditis in a life-long experience of India, Malaya, South China and Central Africa. He also cited MacKinnon, according to whom chorea was never observed in East African children, though Chesterman found this disease occasionally in Central Africa.

There are a few reports in which the occurrence of acute rheumatic fever in the tropics is mentioned. Denecke<sup>7</sup> mentioned the occurrence of the sequelae of rheumatic carditis in Fernando Po (off West Africa) and Rio Muni, though it is not stated how this diagnosis was made. He did not observe scarlet fever. In the Netherlands East Indies clinical acute rheumatic fever has been observed a few times; 1 case of mitral endocarditis is mentioned.<sup>8</sup> We did not find autopsy reports.

Statements to the effect that certain diseases do not or only rarely occur in tropical countries must always be viewed with a certain suspicion. Statistics based on clinical observations alone are unreliable. Only when autopsies with histologic examinations are regularly performed in sufficient numbers and when the eventual differences between the general and the hospital population are taken into consideration, can the incidence of certain diseases in the population of a given region be determined approximately.

1. MacCallum, W. G.: *A Textbook of Pathology*, ed. 7, Philadelphia, W. B. Saunders Company, 1941, p. 788.

2. Boyd, W.: (a) *The Pathology of Internal Diseases*, ed. 4, Philadelphia, Lea & Febiger, 1944, p. 10; (b) *A Text Book of Pathology*, ed. 4, *ibid.*, 1943, p. 154.

3. Forbus, W. D.: *Reaction to Injury*, Baltimore, Williams & Wilkins Company, 1943, p. 231.

4. Steinbröcker, O.: *Arthritis in Modern Practice*, Philadelphia, W. B. Saunders Company, 1942, p. 120.

5. Hegler, C.: *Der acute Gelenkrheumatismus*, in von Bergmann, G., and Staehelin, R.: *Handbuch der inneren Medizin*, ed. 3, Berlin, Julius Springer, 1934, vol. 1, p. 159.

6. Strong, R.: *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*, ed. 6, Philadelphia, The Blakiston Company, 1942, vol. 2, p. 1605.

7. Denecke, K.: *Arch. f. Hyg.* **126**:331, 1941; abstracted, *Trop. Dis. Bull.* **39**:348, 1942.

8. Hidayat, D.: *Geneesk. tijdschr. v. Nederl.-Indië* **72**:1548, 1932. Groot: *ibid.* **76**:1588, 1936.

This applies not only to cancer<sup>9</sup> but also to other diseases, especially when the etiologic factor, as in acute rheumatic fever, is unknown and when the diagnosis can be confirmed only by histologic examination.

As it is the purpose of this paper to demonstrate that rheumatic carditis is not so rare among the natives of Curaçao, a tropical island, some facts about the geographic position and climate of this island are not out of place. Curaçao lies in the Caribbean Sea between 12° 2' 18" and 12° 23' 30" northern latitude. The shortest distance between the island and the coast of South America (Venezuela) amounts to 38 sea miles. The yearly mean temperature is 81.5 F. The average annual rainfall is 21.7 inches (55 cm.), though annual rainfalls of 40 inches (101.5 cm.) and more have been recorded. The rainy season includes the months of October, November, December and January. The relative humidity is ± 73 per cent. In the neighboring islands Aruba and Bonaire the same climatic conditions prevail.<sup>10</sup>

Clinically, acute rheumatic fever is not infrequently observed among the native population of Curaçao.<sup>11</sup> Among 3,391 admissions for internal diseases during the years from 1940 to 1945 there were 61 for acute rheumatic infection. In 20 cases there was acute arthritis, in 30 cases arthritis combined with endocarditis and in 11 cases endocarditis without arthritis (see table). In 3 patients (2 women, 50 and 56 years old and 1 man 40 years old) the disease changed into secondary chronic rheumatism. In the same period 3 cases of typical chorea minor were observed: 2 cases concerned 14 year old girls; one recovered without complications; the other died, and at autopsy endocarditis verrucosa of the mitral valve was found (see report of case 20). The third case concerned a 5 year old girl; four weeks before admission she had suffered an attack of acute rheumatic fever; she died of cardiac decompensation.

Streptococci are regularly isolated from throat swabs; of 124 cases in which throat swabs were examined in the bacteriologic laboratory of the Public Health Service (Dr. A. W. Pot), hemolytic streptococci were found in 40, anhemolytic streptococci in 9 and Streptococcus viridans in 9. We did not observe scarlet fever.

9. Bonne, C.: (a) Am. J. Cancer **25**:811, 1935; (b) **30**:435, 1937. (c) Hartz, P. H.: ibid. **40**:355, 1940.

10. Realino, F. M.: De Nederlandsche Antillen, ed. 3, Curaçao, St. Thomas College, 1938.

11. van der Sar, A.: Rev. Polyclin. Caraças **12**:213, 1943.

Since February 1936 1,481 persons have been examined post mortem on Curaçao. This number includes 174 Chinese, Syrians, Dutch and other nationals; the remainder were in the main natives of Curaçao and a small number of Aruba and Bonaire, where, as already stated, the same climatic conditions prevail. Among these 1,307 autopsies there were 20 in which a gross diagnosis of rheumatic carditis or of the sequelae of rheumatic carditis was made. In 12 of these a histologic examination could be made, and in 11 out of these 12 instances typical Aschoff bodies were found.

#### REPORT OF CASES

1. Negro girl, 12 years old. Clinical data were not available. The autopsy diagnosis was: verrucous endocarditis of the mitral valve; cardiac hypertrophy and dilatation; passive congestion of the lungs and of the liver. The heart weighed 325 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

2. Negro girl, 9 years old. Clinical data were not available. The autopsy diagnosis was: verrucous endocarditis of the mitral valve; myocarditis; fibrinous pericarditis. Microscopic examination of the heart showed typical Aschoff bodies in the myocardium and the pericardium.

3. Negro woman, 24 years old, who died during transport to the hospital. The autopsy diagnosis was: mitral stenosis; chronic inflammation of the mitral and aortic valves; dilatation of the left atrium; thrombosis of the right auricle. The heart weighed 250 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

4. Negro girl, 9 years old, with a history of acute rheumatic fever (polyarthritis). The autopsy diagnosis was: verrucous endocarditis of the mitral valve; fibrinous pericarditis; cardiac hypertrophy and dilatation; thrombosis of the right auricle; brown induration of the lungs. The heart weighed 245 Gm. Microscopic examination of the heart showed Aschoff bodies.

5. Negro boy, 15 years old. Clinical data were not available. The autopsy diagnosis was: endocarditis of the mitral and aortic valves; myocarditis; thrombosis of the right auricle; cardiac hypertrophy and dilatation; adhesive pericarditis; passive congestion of the lungs and of the liver. The heart weighed 365 Gm. Microscopic examination of the heart showed typical Aschoff bodies, especially in the right auricle.

6. Negro boy, 8 years old with a history of acute rheumatic fever (polyarthritis). The autopsy diagnosis was: chronic inflammation of the mitral valve; cardiac hypertrophy and dilatation. The heart weighed 210 Gm. Microscopic examination of the heart showed typical Aschoff bodies in the myocardium and the pericardium.

7. Negro girl, 6 years old, with no history of acute rheumatic fever (polyarthritis) or tonsillitis. On admission an electrocardiogram revealed a P-R interval of 0.16 second; the P-R waves were not enlarged or doubled. During her stay in the hospital a 2:1 block developed, which after a few days was replaced by an intraventricular block; the duration of QRS was 0.12 second. The autopsy diagnosis was: endocarditis verrucosa of the mitral valve; myocarditis; fibrinous pericarditis; cardiac hypertrophy and dilatation; passive

congestion of the internal organs. Microscopic examination of the heart showed large Aschoff bodies in the myocardium and the pericardium.<sup>11a</sup>

8. Negro girl, 9 years old, with acute rheumatic fever (polyarthritis) on admission and in the history. Cultures were made from the blood and from throat swabs, but no streptococci were found. The autopsy diagnosis was: serofibrinous pericarditis, the pericardial sac containing 300 cc. of exudate; chronic endocarditis of the mitral valve; passive congestion of the lungs. The heart weighed 255 Gm. Microscopic examination of the heart showed typical Aschoff bodies, especially in the right auricle.

9. Arubian girl, 17 years old, with no history of acute rheumatic fever. She was admitted because of pulmonary tuberculosis. The autopsy diagnosis was: pulmonary tuberculosis; tuberculous peritonitis; caseous salpingitis; verrucous endocarditis of the mitral and aortic valves; small myocardial scars. Microscopic examination of the heart showed a few typical Aschoff bodies.

10. Negro girl, 10 years old. On admission she had had typical acute rheumatic fever for two days. There

with shortness of breath and retrosternal pain. For two days he had suffered from fever and painful joints. There was marked dilatation of the heart; the teleoentgenogram gave a diameter of 195 mm. There were systolic and diastolic murmurs. The blood culture gave a negative result, and no streptococci could be isolated from a throat swab. Death occurred seven days after admission. The father of the patient had suffered from rheumatic fever at the age of 11 years. From the throats of the father (now 49 years old) and of 3 of 4 brothers and sisters of the patient (respectively, 3, 5 and 18 years old) anhemolytic streptococci were isolated; not, however, from the throat of the sister who died of acute rheumatic fever. The autopsy diagnosis was: fibrinous pericarditis; recurrent verrucous endocarditis of the mitral and aortic valves; mitral insufficiency; marked cardiac hypertrophy and dilatation, especially on the left side. The heart weighed 430 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

12. Arubian woman, 44 years old, who died after a supravaginal hysterectomy, with the symptoms of pulmonary embolism and infarction. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; mitral stenosis; dilatation of the left atrium and of the right ventricle; small myocardial scars;

#### Cases of Acute Rheumatic Infection in Curaçao 1940 to 1945

Distribution of Cases by Age and Sex

Diagnosis	1-5 Yr.	6-10 Yr.	11-15 Yr.	16-20 Yr.	21-25 Yr.	26-30 Yr.	31-35 Yr.	36-40 Yr.	41-45 Yr.	46-50 Yr.	51-55 Yr.	56-60 Yr.	Male	Female	Total	Deaths
acute rheumatic fever (arthritis)	..	1	2	2	2	4	2	1	..	1	4	1	13	7	20	0
acute rheumatic fever (arthritis) and endocarditis	2	6	8	1	1	5	1	2	1	1	1	1	13	17	30	9
Endocarditis.....	1	..	..	6	..	1	2	..	1	..	..	..	3	8	11	5
Total.....	3	7	10	9	3	10	5	3	2	2	5	2	29	32	61	
Deaths.....	2	4	2	2	..	2	..	..	1	..	..	1	7	7		14

was no history of rheumatic fever in the family. There had been typical symptoms of mitral insufficiency. An electrocardiogram showed a P-R interval of 0.22 second, P waves of normal interval and amplitude, and a QRS complex of normal duration and form. The blood culture was negative; a culture of material from the throat gave growths of an anhemolytic streptococcus and a pneumococcus; a culture from a throat swab at a later date gave exclusively a growth of an anhemolytic streptococcus. The autopsy diagnosis was: endocarditis verrucosa of the mitral, tricuspid and aortic valves; myocarditis; cardiac hypertrophy, especially of the left ventricle. The heart weighed 275 Gm. Microscopic examination of the heart showed typical Aschoff bodies in great numbers and numerous small necroses in the papillary muscles of the left ventricle.

11. Negro boy, 12 years old. He was first admitted in June 1943, one day after the appearance of the first symptoms and two days after his sister died of rheumatic fever, chorea minor and mitral insufficiency. He was discharged in fairly good condition after two and a half months. He was readmitted in May 1945

11a. Since this paper was submitted a 7 year old brother of this patient has died. Examination revealed rheumatic carditis with numerous Aschoff bodies.

pulmonary embolism and infarction. The heart weighed 470 Gm. No Aschoff bodies were found at microscopic examination.

In the following cases no tissue was available for microscopic examination.

13. Negro woman, 23 years old. The autopsy diagnosis was: chronic endocarditis of the mitral valve; myocarditis; hypertrophy of the left ventricle.

14. Negro man, 20 years old. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; serofibrinous pericarditis; hypertrophy and dilatation of the left ventricle; brown induration of the lungs.

15. Negro woman, 26 years old, whose death occurred one day after delivery of her infant. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; mitral stenosis, hypertrophy and dilatation of the right ventricle and of the left atrium.

16. Negro woman, 30 years old. The autopsy diagnosis was: chronic endocarditis of the mitral valve; mitral stenosis; verrucous endocarditis of the tricuspid valve; dilatation of the left atrium and of the right ventricle; hypertrophy of the right ventricle.

17. Negro woman, 42 years old. The autopsy diagnosis was: recurrent endocarditis of the mitral valve;

mitral stenosis; dilatation of the left atrium, the right atrium and the right ventricle; brown induration of the lungs.

18. Negro woman, 26 years-old, whose death occurred twenty minutes after delivery. The autopsy diagnosis was: chronic endocarditis of the mitral valve; dilatation of the left atrium; hypertrophy of the right ventricle.

19. Negro girl, 19 years old. The autopsy diagnosis was: fibrinous pericarditis; chronic endocarditis of the mitral and tricuspid valves; myocarditis.

20. Negro girl, 14 years old, with chorea minor. The autopsy diagnosis was: hemopericardium; fibrinous pericarditis; chronic endocarditis of the mitral valve; cardiac hypertrophy. The heart weighed 360 Gm.

#### COMMENT

Although the etiologic nature of acute rheumatic fever is still an unsolved problem despite the several theories which are being discussed, there exists a characteristic lesion, the Aschoff body, by which rheumatic carditis can be recognized with great certainty.<sup>12</sup> Its occurrence in patients in whom a rheumatic infection could be excluded has never been conclusively demonstrated.<sup>12</sup> We believe, therefore, that by the finding of the typical Aschoff bodies the diagnosis of rheumatic carditis has been sufficiently established in cases 1 to 11. In case 12 no Aschoff bodies were found, and in cases 13 to 20 no tissue was available for microscopic examination. Nevertheless a rheumatic origin for the cardiac lesions observed in these cases at autopsy is very probable. These lesions were all typical of rheumatic carditis. The fact that no Aschoff bodies were found in case 12 does not exclude rheumatic fever; these bodies are finally transformed into scar tissue, which in this case is the more probable since the patient was already in the fifth decade of life; moreover, Aschoff bodies may be absent in rheumatic fever.<sup>2</sup> Applicable to cases 12, 16 and 17 is the opinion of Carey Coombs<sup>13</sup> that all cases of mitral stenosis are rheumatic in origin. Furthermore, in view of the high percentage (90 per cent) in which Aschoff bodies were found in the first 12 cases, it is reasonable to suppose that microscopic examination would have yielded several more cases with these bodies, especially among those in which acute pericarditis and verrucous endocarditis were present at autopsy.

It follows from the foregoing report of cases that rheumatic carditis does occur on Curaçao, a tropical island in the Caribbean Sea, and that its occurrence is certainly not rare. Apart from the negative statements from Puerto Rico we

could not obtain data as to the occurrence of this disease on other Caribbean islands. It is however, interesting to note that Jaffé<sup>14</sup> only twice observed rheumatic carditis in his large autopsy material in Caracas (Venezuela).

Several other facts have to be noted: Nearly all of our patients belonged to the poorer classes whose intake of vitamin C is certainly not high though they did not show any symptoms of avitaminosis and we never observed scorbutic conditions in our clinical material; on Curaçao the incidence of acute rheumatic fever does not parallel that of scarlet fever, which is really exceedingly rare on Curaçao, when it occurs at all; different kinds of streptococci are being isolated from throat swabs, but only rarely from the throats of patients suffering from acute rheumatic fever; cultures of blood from the patient were always negative. In 1 case<sup>9a</sup> there was marked familial incidence of the disease.

The conclusion to be drawn from our observations is that statements concerning the rareness of rheumatic carditis in the tropics are probably too general; it is to be expected that when reliable data, especially reports of autopsies with microscopic examinations, become available acute rheumatic fever and rheumatic carditis will be found to occur more frequently in other tropical regions. It is of course possible that tropical countries exist where acute rheumatic fever and rheumatic carditis are really rare or where they do not occur<sup>15</sup>; we believe, however, that in this case the rareness or nonoccurrence of these diseases is not due to the tropical condition per se but to local circumstances or perhaps racial peculiarities, which also play an important role in the incidence and course of other diseases. It must be considered possible that the incidence of acute rheumatic fever is far less influenced by climatic factors than is commonly stated. Theories based on the alleged parallelism between the incidence of scarlet fever and that of acute rheumatic fever are not in accordance with the facts. Finally, physicians sending patients with rheumatic carditis to a tropical country should try to obtain reliable information about the incidence of rheumatic carditis in that country and not rely on climatic data alone.

#### SUMMARY

Among 3,391 admissions for internal disease at Curaçao, Netherlands West Indies, over

14. Jaffé, R.: Personal communication to the author.

15. Rogers, L., and Megaw, J. W. D.: Tropical Medicine, ed. 5, London, J. & A. Churchill, Ltd., 1944, pp. 480 and 481.

12. Saphir, O.: Arch. Path. 32:1000, 1941.

13. Carey Coombs, cited by Boyd.<sup>2a</sup>

period of five years, there were 61 for acute rheumatic fever. Three cases were complicated by chorea minor. Scarlet fever was not observed. Among 1,307 autopsies on natives of Curaçao, Aruba and Bonaire there were 20 which disclosed typical gross lesions or sequelae of rheumatic carditis. In 12 of these histologic examina-

tion was possible, and in 11 typical Aschoff bodies were found.

The supposition is advanced that rheumatic carditis is more frequent in the tropics than is commonly believed but that the true incidence can be determined only by collecting reliable data, based especially on autopsies with histologic examinations.

## FAT NECROSIS STUDIES

### VI. The Effect of Feeding Lipase-Containing Vegetable Seed on the Production of Fat Necrosis

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SOME features of fat necrosis remain baffling. This is largely due to the fact that the lesion occurs under variable conditions and in different locations. It is best considered under the terms: (1) "spontaneous," (2) "experimental," (3) "pancreatic" and (4) "traumatic" or "subcutaneous." The first two terms are self explanatory, and either one is frequently combined with one of the other terms for better designation.

Spontaneous pancreatic fat necrosis may be caused by any process or occurrence that enables pancreatic juice to escape from its natural channels. It does not occur by absorption of that lipase-containing substance from these normal passages. It has followed trauma, acute inflammation and cancer of the pancreas; inflammation, tumor and parasitization of the pancreatic ducts; lithiasic, inflammatory, parasitic or neoplastic obstruction of the ampulla of Vater. The lesions are found within the abdominal fatty tissues unless the enzyme gains access directly to other fat deposits, such as those in the subcutaneous tissue, by way of wounds or drainage tracts.

Experimental fat necrosis has been produced by ligation of the pancreatic ducts, temporary obstruction of the pancreatic vascular circulation, sectioning of the pancreas, placing of fresh pancreatic tissue into the abdominal cavity, and intraperitoneal injections, in both cold-blooded and warm-blooded vertebrates, of fresh pancreatic juice, emulsions of pancreas of various animals, commercial pancreatin and lipase derived from both animal and vegetable sources.<sup>1</sup>

Traumatic or subcutaneous fat necrosis is a fatty tissue change which has the characteristics of pancreatic fat necrosis but results from mechanical trauma of subcutaneous adipose tissue. It is seen principally in the fat of the female mammary gland and in the subcutaneous fat of the newborn. In the latter it is incident to delivery and has been termed sclerema neonatorum. The trauma is responsible for cell death and the consequent liberation of cell-contained lipase, which acts locally

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From the Department of Pathology, Bacteriology and Preventive Medicine,  
University of Missouri School of Medicine.

1. Neal, M. P., and Ellis, M. M.: South. M. J. 23:313, 1930.

on fat. Farr<sup>2</sup> recorded that by pinching the skin and subcutaneous tissue of young pigs with forceps he was able to produce this type of fat necrosis and stated that no pancreatic disease or injury is necessary.

Some writers have been wont to explain the occasional finding of fat necrosis in pericardial, mediastinal, mammary and subcutaneous adipose tissue as of pancreatic origin. They have theorized that lipase of pancreatic source is transplanted by the blood to these sites, where, for an unexplained reason, it acts. One logically asks, Why the particular localization? Why not a more general involvement of fatty tissue, and why do other fat tissues escape?

While the traumatic or subcutaneous type of fat necrosis is of interest and creates a problem in itself, this article is concerned primarily with fat necrosis of the so-called pancreatic origin, meaning that it is the result of the escape of lipase from some portion of the normal passage-way for pancreatic secretion or the introduction of an active lipase-containing substance into living animals.

#### SPONTANEOUS FAT NECROSIS IN ANIMALS

Horvath and Chang<sup>3</sup> reported that in rabbits after prolonged feeding and overconsumption of lipase-containing raw soybeans, they discovered in the perirenal fat, on killing the animals, a lesion which was interpreted as fat necrosis. Soybeans heated until the lipase had been destroyed, according to the ethyl butyrate test, fed to like animals and in similar quantities gave no such lesions in fat tissue. This led to the conclusion that the substance activating the fat tissue change was destroyed by heat. Horvath<sup>4</sup> had previously noted similar necrotic areas in the perirenal fat and sometimes in the subpleural fat of beef cattle in northern China that had been fed heavily on soybean cake and black soybeans (observed in the slaughter house in Tsingtau, China). The lesions in both the rabbits and the beef cattle were interpreted as fat necrosis, and the fact that rabbits fed on cooked soybeans did not show the necrosis was suggestive of an enzyme as the cause. Horvath<sup>4</sup> did not observe the condition in Mongolian cattle fattened on grass (examined in Tientsin).

Horvath and Chang<sup>3</sup> gave no detailed description of the lesion or statement as to the criteria on which they arrived at the conclusion "fat necrosis." There is nothing in their report to indicate histologic, bacteriologic or parasitologic studies of the material.

It is difficult to accept the idea of lipase consumed as a food being removed from the gastrointestinal tract to remote fat deposits and there localized as the factor in these lesions that have been recorded

2. Farr, C. E.: Ann. Surg. 77:513, 1923.

3. Horvath, A. A., and Chang, H. C.: Am. J. Physiol. 78:224, 1926.

4. Horvath, A. A., cited by Horvath and Chang,<sup>3</sup> p. 232.

as fat necrosis. Lipase is present and available in the pancreatic secretions that enter the duodenum. Certainly if it were absorbable from feeding sources, as from lipase-containing soybeans or peanuts, in sufficient quantities to cause fat necrosis, it would be absorbable also from the pancreatic source.

Lipase has an affinity for fat tissue, but one cannot explain why the foci of fat necrosis should be localized, for instance, in the perirenal or some other area. The characteristic fat necrosis occasionally seen in subcutaneous areas and in the breast is to be attributed not to localization of lipase derived from the intestinal tract but to liberation of lipase from cells in the area where the necrosis occurs as a result of some local damage, as that caused by trauma.

*Differential Diagnosis.*—It is well known among pathologists that foci of fat necrosis are readily confused with, and must be differentiated from, foci of tuberculosis, syphilis and cancer, from reactions to certain parasites, from foci of infection, from lesions incident to the injection of oils or following trauma and particularly from foci of postmortem change due to enzyme action on fatty tissues near the pancreas. Caution is especially called for in the interpretation of small white spherical lesions in animals where parasites are common. Neal,<sup>5</sup> in examining the carcasses of hogs that had been fattened on raw peanuts, found parasites of the species *Stephanurus dentatus*, commonly known as the kidney worm of swine, in numbers of these animals. Of particular significance was their presence in the pancreas and the pancreatic duct where, by occluding the duct system, they served as a cause of stagnation. Small lesions, 2 to 4 mm. in diameter, produced by the presence of these parasites or of their ova were on gross inspection differentiated with difficulty from fat necrosis. They were especially found in the peripancreatic, perirenal and periureteral fat deposits. The distinguishing and differential feature was that the parasites or their ova were present within the necrotic-appearing foci. Effort has been made, but without success, to develop a chemical stain that when applied to the gross lesion would give a differential or a selective reaction such as that used in demonstrating amyloid degeneration.

#### PROBLEM

The present studies were undertaken because it had been claimed<sup>6</sup> that the feeding of rabbits and cattle on raw soybeans produced fat necrosis and because our previous efforts<sup>7</sup> at producing this condition

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5. Neal, M. P.: South. M. J. **34**:153, 1941.

6. Horvath and Chang.<sup>8</sup> Horvath.<sup>4</sup>

7. Neal, M. P., and Ellis, M. M.: Am. J. Clin. Path. **1**:251, 1931; J. Missouri M. A. **32**:37, 1935; footnote 1.

in experimental animals had failed except when concentrated and purified lipase, pancreatic secretion, emulsified fresh pancreas or pancreatin was injected either subcutaneously or intraperitoneally.

#### PROCEDURE

*First Experiment* (feeding of rabbits on soybeans or peanuts<sup>8</sup>).—A group of 16 rabbits was fed exclusively for periods of from five to twenty days on lipase-containing, American-grown, Manchu type raw soybeans or Virginia grown raw peanuts, with 8 animals being fed one or the other product. Sixteen rabbits as controls were fed similar products that had been heated to a temperature of 120 C. for one hour to destroy the lipase, and 8 others were fed a regular diet, largely of green vegetables, alfalfa, oats and shorts. At designated five, ten, fifteen and twenty day periods, members of each group and their respective controls were killed and examined.

*Second Experiment* (a study of the carcasses of 8,324 peanut-fattened hogs<sup>5</sup>).—This study was conducted at two southern Georgia packing plants at the height of the seasonal peanut fattening period on animals that were self fed, pastured or grazed on mature peanuts in the field. It was impossible to ascertain the length of time that an individual hog might have been on this type of feed, but it varied from two or three weeks to four months, certainly adequate time for fat necrosis to develop. The animals varied in live weight from 60 to 600 pounds (27 to 272 Kg.) and in ages from 5 or 6 months to 2½ to 3 years (a few). The average was between 150 and 200 pounds (68 and 90.5 Kg.) and between 7 and 10 months of age. They were almost invariably slaughtered within from forty-eight to seventy-two hours after being taken from the farm and their place of feeding, and the entire carcass was personally and immediately inspected. If there was doubt concerning the nature of a gross lesion, histologic studies were consistently done.

*Third Experiment* (feeding of white rats on peanuts or soybeans).—Sixty grown white rats were employed in this study. Thirty were fed exclusively on unshelled, raw Virginia-grown peanuts and 30 on uncracked, raw, American-grown, Manchu type soybeans for periods of time varying from five to ninety-one days. Two animals of each group were killed and minutely examined for evidence of fat necrosis on each of the following days of the period of restricted feeding: fifth, eleventh, fifteenth, twentieth, twenty-fifth, twenty-ninth, thirty-fifth, fortieth, forty-fifth, fiftieth, fifty-fifth and sixtieth. One rat of each group was killed on each of the following days: sixty-fifth, seventieth, seventy-fifth, eighty-second, eighty-seventh and ninety-first.

Immediately after the rats were killed, the various subcutaneous, abdominal and intrathoracic fatty tissues were minutely examined for fat necrosis. Of the 60 rats, all lived until the predetermined date for killing, and all except 7 revealed abundant fatty tissues. The 7 were poor in fat deposits.

The times selected for killing rabbits and white rats were chosen because of knowledge previously acquired from studies on intraperitoneal and subcutaneous injections of lipase in various vertebrates. In these, grossly recognizable foci of fat necrosis were demonstrated as early as sixteen and a half hours and as late as eight days after single injections of the lipase extractives.<sup>1</sup>

8. Neal, M. P.: Fat Necrosis Studies: V. The Effect of Soybean and Peanut Feeding on the Blood and Urine Lipase of Rabbits and in the Production of Fat Necrosis, South. M. J. 38:793, 1945.

## RESULTS

Of the 16 rabbits fed exclusively on raw soybeans or raw peanuts, those killed on the fifth, tenth, fifteenth and twentieth days showed no fat necrosis.

Among 8,324 peanut-fattened hogs, only 3 disclosed perirenal and 2 pancreatic fat necrosis. The presence of the kidney worm, *Stephanurus dentatus*, in the pancreas or the pancreatic duct, and within the kidney or the perirenal fat, was considered the cause of fat necrosis in these 5 hogs.

Thirty white rats fed exclusively raw peanuts and 30 others fed raw soybeans for periods of from five to ninety-one days failed to show fat necrosis when some of each group were put to death at essentially five day intervals.

## SUMMARY

Under the conditions elsewhere described<sup>8</sup> fat necrosis is not produced in rabbits by the feeding of lipase-containing peanuts or soybeans.

Under the conditions of fattening, marketing and slaughtering of hogs, as previously reported,<sup>5</sup> fat necrosis is not produced by the feeding of lipase-containing peanuts. Fat necrosis was not produced in 60 white rats fed lipase-containing peanuts or soybeans.

Reports indicating that the ingestion of lipase-containing food, notably soybeans, is capable of producing fat necrosis in animals have not been confirmed by these studies. In fact, the evidence is that lipase in food may be discounted as a cause of fat necrosis.

Postmortem fatty tissue changes and those resulting from trauma, parasitization, infection and pancreatic lesions must be eliminated as factors before a food source of lipase is acceptable as producing this condition.

Spontaneous fat necrosis arising from conditions other than those that permit leakage of lipase from the normal pancreatic channels is also to be considered as the result of lipase but of lipase derived from some source other than that which enters the body by the way of the alimentary tract; i. e., lipase contained within food or forming a part of a food.

Interpretations based on gross inspection of the lesions alone are not dependable.

## EXPERIMENTAL NEPHROPATHIES

### IV. GLYCOSURIA IN DOGS POISONED WITH URANYL NITRATE, MERCURY BICHLORIDE AND POTASSIUM DICHROMATE

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Phosphatase is abundant in the superficial layers of the upper part of the intestine<sup>1</sup> and in the proximal convoluted tubules of the kidneys.<sup>2</sup> It is absent from the mucosa of the rectum and from the nephron from Henle's loop onward.<sup>2</sup> Glucose is absorbed rapidly from the duodenum and the jejunum<sup>3</sup> and in the proximal convoluted tubules of the kidneys.<sup>4</sup> It is absorbed very slowly from the rectum<sup>5</sup> and probably not at all in Henle's loop and the distal convoluted tubules. Numerous investigators<sup>6</sup> have therefore considered the possibility that renal phosphatase takes part in the absorption of glucose from the glomerular filtrate. Wilmer<sup>7</sup> has recently described a theoretic mechanism by which this enzyme may play a role in the absorption of sugar in the kidneys.

Although the literature dealing with the renal effects of the three inorganic chemical agents used in these experiments is extensive, relatively few investigators have included the possible production of glycosuria as one of the results. In 1913 Frank<sup>8</sup> injected subcutaneously into 5 rabbits 4 to 10 mg., and into 5 dogs 10 to 35 mg., of uranyl nitrate ( $\text{UrO}_2[\text{NO}_3]_2$ ) and observed glycosuria in all of them. He injected intravenously into 7 rabbits 0.4 to 4.0 mg. of mercury bichloride ( $\text{HgCl}_2$ ) and observed glycosuria in all of these animals. One dog given 50 mg. of potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) by subcutaneous in-

jection had 2 plus sugar in its urine. Milhorat and Deuel<sup>9</sup> produced glycosuria by injecting 15 mg. of uranyl nitrate per kilogram into each of 12 dogs, although the blood sugar levels in these animals never exceeded those in the normal controls. Both Frank<sup>8</sup> and Milhorat and Deuel<sup>9</sup> used much larger doses of the poisons than were employed in our experiments, which appear to stand alone as an attempt to determine the smallest dose capable of causing excretion of sugar in the urine. Major<sup>10</sup> studied fatal "chromic acid" poisoning in a human patient and observed that glycosuria appeared from time to time but that it bore no apparent relation to the amounts of blood sugar present. Mosenthal and Schlayer<sup>11</sup> reported glycosuria after poisoning with potassium dichromate.

#### RESULTS OF EXPERIMENTAL STUDY

The methods employed in these experiments have been described in a previous paper in this series.<sup>12</sup> Benedict's solution was used in testing the urine for sugar.

The urines of 54 dogs in our series<sup>12</sup> were examined for the presence of sugar after poisoning with various chemical agents, distributed as follows:

Uranyl nitrate .....	15 dogs
Mercury bichloride .....	17 dogs
Potassium dichromate .....	17 dogs
Phlorhizin .....	5 dogs
Total.....	54 dogs

The relation of the presence or the absence of glycosuria to other chemical and microscopic observations is presented in table 1.

There is a marked difference in the incidence of glycosuria in dogs poisoned with the three inorganic chemical agents used in these experiments. Two thirds of the dogs poisoned with uranyl nitrate had glycosuria, while this condition was found in less than one fourth (23.5 per cent) of those poisoned with mercury bichloride.

9. Milhorat, A. T., and Deuel, H. J.: Arch. Int. Med. **60**:77, 1937.

10. Major, R.: Bull. Johns Hopkins Hosp. **33**:56, 1922.

11. Mosenthal, H. O., and Schlayer: Deutsches Arch. f. klin. Med. **111**:127, 1913.

12. Simonds, J. P., and Hepler, O. E.: Arch. Path. **39**:103, 1945.

From the Department of Pathology, Northwestern University Medical School.

1. Grosser, P., and Husler, J.: Biochem. Ztschr. **39**:1, 1912.

2. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

3. MacKay, E. M., and Clark, W. G.: Am. J. Physiol. **135**:187, 1941.

4. Smith, H. W.: The Physiology of the Kidneys, New York, Oxford University Press, 1937.

5. Houssay, B. A.; Foglia, V. G., and Fustinoni, O.: Endocrinology **28**:915, 1941.

6. (a) Dmochowski, A., and Assenhajm, D.: Naturwissenschaften **23**:501, 1935. (b) Kutscher, W., and wissenschaften **23**:559, 1935. Wolbergs, H.: ibid. **23**:559, 1935.

7. Wilmer, H. A.: Arch. Path. **37**:227, 1944.

8. Frank, E.: Arch. f. exper. Path. u. Pharmakol. **72**:387, 1913.

ride and potassium dichromate. The difference is even more pronounced when one compares the minimum dose of each agent required to produce glycosuria. Of 10 dogs that received single doses of 1 mg. or more of uranyl nitrate per hundred cubic centimeters of blood, 100 per cent had glycosuria. Two doses of 1 mg. each of potassium dichromate per hundred cubic centimeters of blood constituted the minimum amount of dichromate required to induce glycosuria. But even this was not constant, for only 4, or 44.4 per cent,

For technical reasons it became necessary with some animals to take blood from the inferior vena cava with the animal under ether anesthesia at the time the animal was killed. As was expected, the sugar content of each of these samples was very high. In table 2 a comparison is made between the blood sugar values before and after poisoning in those dogs from which blood was taken by a leg vein only. Within the limitation of this small group of 20 dogs, uranyl nitrate caused an increase in blood sugar with and without

TABLE 1.—Glycosuria in Dogs Poisoned with Uranyl Nitrate ( $\text{Uro}_2[\text{NO}_3]_2$ ), Mercury Bichloride ( $\text{HgCl}_2$ ), Potassium Dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) and Phlorhizin

Dog	Dose, Mg. per 100 Cc. of Blood	Glyco- suria	Albumi- nuria	Blood Phosphatase, Bodansky Units		Blood Sugar, Mg. per 100 Cc.		Kidney Phosphatase		Necrosis of Tubular Epithelium
				Before	After	Before	After	Chemical Bodansky Units	Micro- scopic	
K Ur 15	1 x 0.05 mg.	0	Trace	3.00	5.00	97.0	174.0*	16.06	++++	0
K Ur 14	1 x 0.10 mg.	0	Trace	0.80	1.00	102.0	143.0*	11.52	+++	0
K Ur 16	1 x 0.10 mg.	0	+	3.00	3.00	74.5	127.0*	17.24	++++	0
K Ur 17	1 x 0.20 mg.	0	+	2.60	4.00	77.5	124.0	13.58	++++	0
K Ur 9	2 x 0.50 mg.	0	+	2.00	3.30	78.5	76.0	10.40	++++	0
K Ur 10	1 x 2.00 mg.	+	+++	2.70	2.20	82.0	112.0	15.04	++++	++
K Ur 12	1 x 2.00 mg.	+	+++	4.60	8.20	62.5	106.0	14.77	+++	++++
K Ur 20	2 x 3.00 mg.	+	+++++	....	....	....	....	14.44	++++	+++++
K Ur 21	2 x 3.00 mg.	+	+++++	....	....	....	....	11.07	++++	+++++
K Ur 22	2 x 3.00 mg.	+	+++++	....	....	....	....	21.70	++++	+++++
K Ur 8	2 x 1.00 mg.	++	+++	3.70	12.80	93.5	85.5	18.85	++++	++
K Ur 13	1 x 2.00 mg.	++	+++	8.70	5.70	83.7	101.0	18.09	+++	+++
K Ur 11	1 x 2.00 mg.	+++	++	3.80	3.10	79.0	112.0	15.21	++++	+++
K Ur 18	1 x 1.00 mg.	++++	++	0.30	2.50	89.0	174.0*	14.15	++++	++
K Ur 19	1 x 1.00 mg.	+++++	++	3.10	101.0	172.0*	15.66	++++	++	++
K Hg 16	1 x 1.00 mg.	0	+	6.83	5.26	....	....	27.12	++++	+
K Hg 17	1 x 1.00 mg.	0	Trace	3.10	2.66	....	....	19.05	++++	+
K Hg 20	2 x 1.00 mg.	0	++	2.33	2.80	....	....	19.11	+++	++
K Hg 21	2 x 1.00 mg.	0	+	4.88	2.75	....	....	21.45	++++	++
K Hg 22	2 x 1.00 mg.	0	++	4.05	5.72	....	....	13.56	.....	+
K Hg 23	2 x 1.00 mg.	0	Trace	4.63	13.33	....	....	18.78	.....	+
K Hg 14	3 x 1.00 mg.	0	....	1.68	2.14	....	....	16.90	+++	+++
K Hg 15	3 x 1.00 mg.	0	....	3.35	3.54	....	....	9.67	++	++
K Hg 18	1 x 2.00 mg.	0	....	4.36	2.85	....	....	13.61	.....	-
K Hg 19	1 x 2.00 mg.	0	....	2.98	2.71	....	....	22.95	+++	++
K Hg 3	3 x 1.00 mg.	0	++	....	....	....	....	6.13	+++	++
K Hg 4	3 x 1.00 mg.	0	++	....	2.74	....	....	7.66	+++	++
K Hg 5	1 x 3.00 mg.	0	++++	4.60	....	....	....	4.28	++	++++
K Hg 7	1 x 3.00 mg.	+	++++	2.92	....	....	....	8.47	+++	++++
K Hg 9	1 x 3.00 mg.	+	++	....	....	....	....	4.99	+++	++++
K Hg 10	1 x 3.00 mg.	+	++	....	....	....	....	5.60	+++	++++
K Hg 6	1 x 3.00 mg.	++	++++	3.38	....	....	....	11.00	-++	+++++
K Cr 9	1 x 0.20 mg.	0	Trace	3.40	1.50	80.0	81.0	18.72	-++	0
K Cr 14	1 x 0.50 mg.	0	....	5.60	3.10	80.5	81.0	17.72	.....	0
K Cr 7	2 x 0.50 mg.	0	++	....	2.90	80.4	126.0*	17.81	++++	0
K Cr 21	2 x 0.50 mg.	0	0	....	....	....	....	.....	.....	0
K Cr 22	2 x 0.50 mg.	0	++	....	....	....	....	.....	.....	0
K Cr 10	1 x 1.00 mg.	0	++	2.30	2.10	92.0	88.0	11.18	++++	+
K Cr 12	1 x 1.00 mg.	0	....	2.60	2.80	77.0	60.0	10.92	.....	+
K Cr 15	1 x 1.00 mg.	0	....	9.50	6.30	75.0	74.0	12.07	+++	+
K Cr 6	2 x 1.00 mg.	0	++	....	4.70	80.7	101.0	12.68	++++	+
K Cr 19	2 x 1.00 mg.	0	++	....	....	....	....	.....	.....	+
K Cr 20	2 x 1.00 mg.	0	++	....	....	....	....	.....	.....	++
K Cr 11	1 x 2.00 mg.	0	++	1.40	1.50	76.0	74.5	18.86	++++	++
K Cr 17	2 x 3.00 mg.	0	++	....	....	....	....	7.40	+++	++++
K Cr 8	2 x 1.00 mg.	+	++	1.40	0.80	94.0	125.0	6.00	+++	++++
K Cr 13	1 x 2.00 mg.	+	....	2.40	1.50	67.0	80.0	8.00	+++	+++
K Cr 16	2 x 3.00 mg.	+	++	....	....	....	....	6.65	+++	+++
K Cr 18	2 x 3.00 mg.	+++	++	....	....	....	....	9.60	+++	+++
K Phl 4	2 x 1.00 mg.	..	....	3.30	3.20	91.0	204.0*	11.00	N	0
K Phl 3	2 x 1.00 mg.	++	Trace	4.30	3.40	56.0	75.5	20.00	N	0
K Phl 1	2 x 0.50 mg.	++++	0	....	....	78.8	69.0	13.00	N	0
K Phl 2	2 x 0.50 mg.	++++	Trace	2.40	3.30	73.5	64.5	21.70	N	0
K Phl 5	2 x 0.50 mg.	++++	0	1.80	1.60	88.0	138.0*	19.73	N	0

\* Blood for determination of sugar was taken from the inferior vena cava while the dog was under ether anesthesia. In the instance of all other dogs blood was drawn from a subcutaneous vein in the leg without anesthesia.

N means normal in amount and in distribution, i. e., in the brush border of the epithelium of the proximal convoluted tubules.

of 9 dogs that received this dose had sugar in the urine. Three milligrams per hundred cubic centimeters of blood constituted the smallest dose of mercury bichloride to cause glycosuria; of 5 dogs that received this dose, only 4, or 80 per cent, had sugar in the urine.

All the dogs in this series that showed glycosuria also had albuminuria, except those receiving phlorhizin. But the converse is not true; that is, many of these dogs had albumin in the urine without glycosuria.

Blood for determination of sugar was taken in most instances from a vein in the leg without anesthesia.

glycosuria, while potassium dichromate caused a rise in blood sugar chiefly in those dogs that had glycosuria. The highest value of sugar in blood from a leg vein was 125 mg. per hundred cubic centimeters, which is well below the threshold of dextrose. After poisoning with phlorhizin, on the other hand, the blood sugar was lower than the control values in the 3 dogs on which we have results. Lundsgaard,<sup>13</sup> Kastler,<sup>14</sup>

13. Lundsgaard, E.: Biochem. Ztschr. **217**:162, 1930.

14. Kastler, A. O.: J. Biol. Chem. **76**:43, 1928.

Duncan<sup>15</sup> and others have reported hypoglycemia following injections of phlorhizin. This is probably due to depletion of the body's supply of carbohydrate by loss through the urine.

No constant relation was apparent between blood phosphatase and glycosuria when the values for dogs with sugar in the urine were compared with the values for dogs without glycosuria. Poisoning with mercury bichloride produces quantitative changes in the phosphatase of the blood as previously reported by us.<sup>16</sup>

TABLE 2.—Glycosuria and Blood Sugar After Poisoning with Nephrotoxic Agents

	Average Value of Blood Sugar, Mg. per 100 Cc.		Dogs with Higher Blood Sugar After Poisoning		
	Before	After	Dogs	No.	Per Cent
UrO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>					
Glycosuria.....	80.0	103.3	5	4	80
No glycosuria....	78.0	100.0	2	1	50
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>					
Glycosuria.....	80.5	102.5	2	2	100
No glycosuria....	80.2	79.9	7	3	43
Phlorhizin					
Glycosuria.....	80.7	69.7	3	0	0
No glycosuria....	91.0	....	1		

The values of blood phosphatase and their relation to glycosuria in this series of dogs are shown in table 3. After the administration of uranyl nitrate, the average value of blood phosphatase in dogs with and without glycosuria was increased, but only 50 and 60 per cent, respectively, of these dogs had higher phosphatase after poisoning with this substance. After, poisoning with mercury bichloride one half of the dogs without glycosuria had higher blood phosphatase than before the poison was administered. Potassium dichromate ap-

TABLE 3.—Glycosuria and Alkaline Blood Phosphatase

	Average Value of Blood Phosphatase, Bodansky Units per 100 Cc.		Dogs with Higher Blood Phosphatase After Poisoning		
	Before	After	Dogs	No.	Per Cent
UrO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>					
Glycosuria.....	3.97	5.37	6	3	50.0
No glycosuria...	2.28	3.26	5	3	60.0
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>					
Glycosuria.....	3.15	4.23	2	?	50.0
No glycosuria...	3.89	3.11	10	5	50.0
Phlorhizin					
Glycosuria.....	2.83	2.77	3	1	33.3
No glycosuria...	3.30	3.20	1	0	0.0

peared to lower blood phosphatase. In 4 dogs blood phosphatase was slightly reduced after injection of phlorhizin. Anderson and Squires,<sup>17</sup> who used larger doses than were employed in our experiments, found that phlorhizin-induced diabetes was accompanied by a decided increase in serum phosphatase.

The relation of renal alkaline phosphatase (stated in Bodansky units) to glycosuria is shown in table 4.

15. Duncan, G. G.: Diseases of Metabolism, Philadelphia, W. B. Saunders Company, 1942, pp. 24 and 694.

16. Hepler, O. E.; Gurley, H., and Simonds, J. P.: Arch. Path. 39:133, 1945.

17. Anderson, R. K., and Squires, R. B.: J. Biol. Chem. 124:71, 1938

The relation of renal phosphatase to glycosuria revealed by Gomori's method in these dogs can be seen in table 1. Among the dogs poisoned with uranyl nitrate, the mean value of renal phosphatase was lower in those without glycosuria but was equal to or greater than the normal mean of  $10.72 \pm 0.33$  in all dogs of both groups.<sup>16</sup> The difference was much greater in the dogs poisoned with mercury bichloride and potassium dichromate. Not only was the phosphatase lower in the dogs with glycosuria than in those without sugar in the urine, but it was 30 per cent below the normal mean of the controls in this series.<sup>16</sup> Among 13 dogs poisoned with mercury bichloride but without glycosuria, renal phosphatase was less than the normal mean in 4, while of the 4 with sugar in the urine, 3 were well below and 1 was within the limits of error of the normal mean. Renal phosphatase in the potassium dichromate dogs averaged 29 per cent below the normal mean in all animals with glycosuria and in only 1 dog without glycosuria. Sections of the kidneys of these dogs stained by Gomori's method<sup>2</sup> revealed phosphatase in the epithelium of the proximal convoluted tubules both in those with and in those without glycosuria. Among the dogs given phlorhizin renal phosphatase was lower in the dog whose urine was not tested for sugar than in the 4 dogs whose urine contained sugar, but it was above the normal mean in all. In sections of the

TABLE 4.—Glycosuria and Alkaline Renal Phosphatase

	Dogs	Mean Value of Phosphatase, Bodansky Units	Standard Deviation
UrO <sub>2</sub> (NO <sub>3</sub> ) <sub>2</sub>			
Glycosuria.....	10	$16.15 \pm 0.45$	$2.12 \pm 0.32$
No glycosuria...	5	$18.76 \pm 0.84$	$2.80 \pm 0.32$
HgCl <sub>2</sub>			
Glycosuria.....	4	$7.51 \pm 0.82$	$2.44 \pm 0.57$
No glycosuria...	13	$15.41 \pm 1.25$	$6.68 \pm 0.87$
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>			
Glycosuria.....	4	$7.56 \pm 0.46$	$1.38 \pm 0.33$
No glycosuria...	9	$13.80 \pm 0.89$	$3.96 \pm 0.63$
Phlorhizin			
Glycosuria.....	4	18.58	
No glycosuria...	1	11.00	

kidneys of the 5 phlorhizin dogs stained by Gomori's method, phosphatase was present in normal amount and in normal distribution.

For 12 dogs of this group blood urea was determined along with sugar in the blood and the urine. There was no constant relation between these two excreted substances. This is in harmony with the observation of Wallace and Myers.<sup>18</sup> Glycosuria was not present in any of the 4 dogs with a blood urea concentration less than 20 mg. per hundred cubic centimeters. However, 1 dog poisoned with potassium dichromate with only 23.4 mg. of urea per hundred cubic centimeters of blood had a high content of sugar in the urine, while another with 61.8 mg. per hundred cubic centimeters did not have glycosuria. In the other 6 dogs of this group the blood urea ranged from 78 mg. per hundred cubic centimeters upward, and all had glycosuria.

#### SUMMARY AND RESULTS

The incidence and the extent of glycosuria in dogs poisoned with chemical agents were compared with other factors determined simultaneously. Some degree of correlation was found

18. Wallace, G. B., and Myers, H. B.: J. Pharmacol. & Exper. Therap. 5:511, 1913-1914.

between glycosuria and (1) tubular necrosis, (2) albuminuria and (3) the chemical agent and its dose. No definite correlation was observed between glycosuria and blood sugar, blood urea, blood phosphatase and renal phosphatase.

All the dogs that had sugar in the urine except those given phlorhizin also had marked albuminuria and extensive necrosis of the tubular epithelium. But the converse was not true. The most constant and definite correlation was found between glycosuria and the chemical agent and the dose used. Uranyl nitrate was the most potent of the three inorganic agents in producing glycosuria, for each dog that received 1 mg. or more per hundred cubic centimeters of blood was found to have sugar in its urine. Two milligrams of potassium dichromate per hundred cubic centimeters of blood were required to produce glycosuria, and this dose was effective in only 44.4 per cent of the dogs. Three milligrams of mercury bichloride produced glycosuria in only 80 per cent of the animals receiving this dose.

Uranyl nitrate appeared to produce a moderate increase in blood sugar in dogs with and without glycosuria; potassium dichromate produced a moderate increase in blood sugar in the dogs with glycosuria but not in those in which the urine was free from sugar; phlorhizin appeared to lower the blood sugar slightly. Uranyl nitrate tended to increase the blood phosphatase, while potassium dichromate and phlorhizin decreased it slightly. In the dogs poisoned with uranyl nitrate, renal alkaline phosphatase was above the normal mean both in those with and in those without glycosuria but was higher in those without sugar in the urine. Among the dogs poisoned with mercury bichloride and potassium dichromate, the mean renal phosphatase was markedly reduced in those with glycosuria and was increased in those without glycosuria. Renal phosphatase was markedly increased in 4 phlorhizined dogs with glycosuria, while in 1 dog for which we have no data concerning sugar in the urine renal phosphatase was within the limits of error of the normal mean.

#### COMMENT

Previous investigators have quite generally concluded (1) that the glycosuria which follows poisoning with chemical agents, particularly with uranium, which has been most extensively studied in this connection, is of renal origin and due to damage of the renal tubules and (2) that it is not related to blood sugar levels.<sup>9</sup> We agree with the first conclusion but we are not

in full accord with the second. There also remain for discussion (1) the differences in power to produce glycosuria possessed by the different chemical agents used in these experiments, (2) the mechanism of the glycosuria and (3) its relation to renal phosphatase.

An observation of Shannon, Farber and Troast<sup>19</sup> is pertinent to the relation between blood sugar levels and glycosuria under the conditions of these experiments. When glucose reabsorption is studied in the normal dog with progressive increments in the plasma arterial concentration of glucose, the plasma level at which frank glycogenesis appears is essentially the same as that at which glucose  $T_m$ , or complete saturation of all the nephrons, is reached. It is likely that there is a variation in the size of the glomeruli and in the length of the renal tubules in any normal animal. These factors are not independent but are coordinated parts of the structural-functional relationships of the nephron as a whole. The higher the filtration rate of any glomerulus in relation to the ability of its tubule to reabsorb glucose, the lower the plasma glucose concentration which is necessary to saturate the reabsorptive capacity of the tubule.

The chemical agents in the dosages employed in these experiments did not produce visible injury in the glomeruli but, except for phlorhizin, they did damage the tubular epithelium in those dogs that had glycosuria. These conditions upset the balance between the correlated capacities of the glomeruli and the tubules—the one to produce filtrate and the other to reabsorb glucose from the filtrate. The reabsorptive capacity of the tubules thus damaged would be completely saturated at a lower plasma glucose concentration than that of a normal animal. Any rise in the plasma glucose level from any cause would therefore make glycosuria a necessary consequence and increase its degree. A normal or even a lowered plasma glucose concentration could, under such conditions, be accompanied by glycosuria.

In view of the statements in the two immediately preceding paragraphs the mechanism of glycosuria in the dogs poisoned with the inorganic substances used in these experiments is relatively simple. The damage to the epithelium of the tubules reduces the glucose  $T_m$  to such an extent that a normal or even a reduced glucose plasma concentration is in excess of that required to saturate all the nephrons. In diabetes mellitus the high plasma glucose oversatu-

19. (a) Shannon, J. S.; Farber, S., and Troast, L.: Am. J. Physiol. 133:752, 1941. (b) Mirsky, I. A., and Nelson, N.: Arch. Int. Med. 71:827, 1943.

rates the normal  $T_m$ ,<sup>19b</sup> in poisoning with these chemical agents the normal plasma glucose more than saturates the reduced  $T_m$ . Glycosuria results in both cases.

It is difficult to explain the difference in power of uranyl nitrate, mercury bichloride and potassium dichromate to produce glycosuria. The difference in dosage required has been mentioned. Glycosuria was clearly dependent on necrosis, but that necrosis was not the sole factor involved is evident on reference to table 1. In that table necrosis is evaluated as 1 plus to 4 plus. Necrosis graded 1 plus involved only an occasional tubule and was limited to the agglomerular, subcapsular zone and to the labyrinth in dogs poisoned with potassium dichromate and to the terminal, straight portion of an occasional proximal convoluted tubule along the margins of the labyrinth in dogs poisoned with mercury bichloride and uranyl nitrate. Necrosis of 4 plus grade involved the greater part of all the tubules. Two plus and 3 plus grades lay between these extremes.

Among the glycosuric dogs poisoned with uranyl nitrate, 3 had necrosis of only 2 plus grade, and the lowest sugar levels were in the dogs with 4 plus necrosis. On the other hand, all the dogs poisoned with mercury bichloride and potassium dichromate that became glycosuric had 3 and 4 plus necrosis. It does not seem possible, therefore, that the low levels of urinary sugar in dogs poisoned with uranyl nitrate could have been due simply to sugar's having diffused through the necrotic material in the tubules into the peritubular capillaries, although the osmotic pressure of the plasma proteins in these capillaries is higher than in any other groups of capillaries in the body because of the loss of water in the glomeruli.

A comparison of the differential effects of these three chemical agents on blood sugar and on

blood and renal phosphatase does not warrant a conclusion that any one of these factors was concerned in the difference in power to produce glycosuria. Differences in the location of the tubular damage with the doses employed in these experiments do not furnish a satisfactory explanation; for uranyl nitrate and mercury bichloride affect the same portion of the proximal convoluted tubule, and yet the former is clearly much more potent in the production of glycosuria than is the latter, which causes a higher degree of calcification.<sup>20</sup> At present, therefore, we have

no explanation for the fact that smaller doses of uranyl nitrate with lower grades of necrosis produce glycosuria with greater constancy and to a more marked degree than do mercury bichloride and potassium dichromate.

In a previous paper<sup>16</sup> we suggested the possibility—and only the possibility—that the mere presence of phosphatase in the brush border of the proximal convoluted tubules is not necessarily proof that it takes part in the functioning of these tubules. Its presence *might* be due to an abortive attempt to reabsorb from the glomerular filtrate a molecule too large for these cells to transmit through their cytoplasm. We found no evidence that this enzyme is concerned with the deposition of calcium in the kidneys of dogs poisoned with the nephrotoxic substances uranyl nitrate, mercury bichloride and potassium dichromate. The experiments here reported show that glycosuria can occur in dogs whose kidneys contain either an abundance of phosphatase (uranyl nitrate and phlorhizin) or a reduced amount of this enzyme (mercury bichloride and potassium dichromate). There is a much closer correlation between glycosuria and necrosis of the proximal convoluted tubules than between glycosuria and the quantity of phosphatase present in the kidneys. As pointed out in previous papers,<sup>21</sup> phosphatase may be abundant in kidneys with extensive necrosis. The large amount of phosphatase in these cases could be due to adsorption of the enzyme by the granular necrotic material in the tubular lumens.

We have been unable to find any constant or provable correlation between alkaline phosphatase activity in the kidneys and any normal or pathologic processes occurring in these organs. The mechanism of the action of alkaline renal phosphatase in the performance of its alleged function is still a hypothesis based on one or more postulates.<sup>22</sup>

During the past decade or more much of the literature which has dealt with the mechanism of reabsorption of glucose by the renal tubules has been concerned with the phosphorylation of this sugar. Martland and Robison,<sup>23</sup> Kay<sup>24</sup> and Lundsgaard<sup>25</sup> expressed the belief that phosphatase is a reversible enzyme capable both of synthesizing and of dephosphorylating hexose phosphate. This was later disputed by Lunds-

21. Footnotes 16 and 20.

22. Kalckar, H.: Chem. Rev. 28:71, 1941.

23. Martland, M., and Robison, R.: Biochem. J. 21:665, 1927.

24. Kay, H. D.: Biochem. J. 22:855, 1928.

25. Lundsgaard, E.: Biochem. Ztschr. 264:209 and 221, 1933.

20. (a) Hepler, O. E., and Simonds, J. P.: Arch. Path. 40:37, 1945. (b) Hepler, O. E.; Gurley, H., and Simonds, J. P.: Proc. Soc. Exper. Biol. & Med. 44:221, 1940.

gaard himself,<sup>26</sup> by Kalckar<sup>27</sup> and by Lipmann.<sup>28</sup> The difficulties associated with the attempt to relate phosphorylation to a synthesizing action of phosphatase have been recently summarized by Kritzler and Gutman.<sup>29</sup> Kalckar<sup>22</sup> and Colowick and co-workers<sup>30</sup> have presented evidence that phosphorylation of glucose occurs as a result of a specific enzyme system, renal phosphorylase, which constitutes a part of the specific cellular mechanisms postulated to explain the rapid transport of glucose from the lumens of the proximal convoluted tubules to the blood stream. Wilmer,<sup>7</sup> using this concept, has presented a theoretic scheme according to which phosphorylase synthesizes hexose phosphate in the tubular epithelial cells; phosphatase then breaks down this compound and liberates the glucose, which is transmitted through the cells to the tissue spaces and thence to the blood in the intertubular capillaries.

Wilmer's interesting theory presents certain mechanical difficulties. Phosphatase is known to be limited to the brush border of the epithelium of the proximal convoluted tubules. The location of the phosphorylase in these cells is not known. Since phosphorylase, according to this theory, must act on glucose first, it should theoretically, be nearer the sugar to be absorbed from the tubular lumens than is phosphatase. This can hardly be true. If the phosphorylating enzyme is also in the brush border, it might be possible for two opposing actions to take place within such a limited space, but it certainly adds difficulties to the acceptance of the hypothesis. If phosphorylase is distributed throughout the cell, it seems likely that it would rephosphorylate glucose that had been dephosphorylated by phosphatase nearer the lumen of the tubule. In our experiments the nephrotoxic substances caused damage and necrosis of the tubular epithelium and diffusion of phosphatase throughout the cell. This would obviously disturb the spatial relations of the two enzymes and interfere markedly with whatever combined function they may perform.

Although many authors refer to the presence of phosphorylase in the kidneys, there appear

to have been few quantitative determinations of the content. Shapiro and Wertheimer,<sup>31</sup> however, have recently made such determinations and concluded that organs with a highly active glycogen metabolism, e. g., skeletal and cardiac muscle and liver, have much more active phosphorylase than do organs such as the lungs, the skin, the testes, the intestines, the spleen, adipose tissue and the placenta, with less active glycogen metabolism. The kidneys and the brain occupy an intermediate position both in phosphorylase activity and in glycogen metabolism. They found that the kidneys converted glucose-1-phosphate into only about 50 per cent of the theoretic maximum polysaccharide. Glycogenolysis in the kidneys amounted to 0.75, compared with about 2.0 for that of skeletal and cardiac muscle and liver.

If phosphatase does take part in renal function it would seem to be a logical hypothesis that it must be concerned with the absorption of glucose from the glomerular filtrate. However, we have found no correlation and no constant relationship between alkaline renal phosphatase and the glycosuria which accompanied poisoning with uranyl nitrate, mercury bichloride and potassium dichromate. But the absence of any such relationship under the conditions of these experiments does not exclude the possibility that this enzyme may function in normal kidneys after the manner described by Wilmer<sup>7</sup> or in some other way. These nephrotoxic substances in adequate doses produce massive necrosis of the tubular epithelium, but active phosphatase is still present in the necrotic material.<sup>16</sup> We do not know what effect these agents have on phosphorylase, the action of which must, theoretically, precede that of phosphatase.

Various chemical substances have been found either to accelerate or to inhibit the action of phosphatase. These were reviewed in a previous paper.<sup>16</sup> There is little available information concerning the effects of chemical agents on phosphorylase. It is said to be inhibited by phlorhizin,<sup>25</sup> by lack of oxygen,<sup>32</sup> by a cyanide,<sup>32</sup> by iodoacetic acid,<sup>33</sup> by traces of copper,<sup>34</sup> by ammonium sulfate<sup>35</sup> and by sodium beta glycro-

26. Lundsgaard, E.: Skandinav. Arch. f. Physiol. **72**:265, 1935.

27. Kalckar, H.: Enzymologia **2**:47, 1937.

28. Lipmann, P., in Nord, F. F., and Werkman, C. H.: Advances in Enzymology, New York, Interscience Publishers, Inc., 1941, vol. 1, p. 99.

29. Kritzler, R. A., and Gutman, A. B.: Am. J. Physiol. **134**:94, 1941.

30. (a) Colowick, S. P.; Welsh, M. S., and Cori, C. F.: J. Biol. Chem. **133**:359, 1940. (b) Colowick, S. P.; Kalckar, H., and Cori, C. F.: ibid. **137**:243, 1941.

31. Shapiro, B., and Wertheimer, E.: Biochem. J. **37**:397, 1943.

32. Footnotes 22 and 27.

33. Wilbrandt, W., and Laszt, L.: Biochem. Ztschr. **259**:308, 1933.

34. Cori, G. T., and Cori, C. F.: J. Biol. Chem. **133**:733, 1940.

35. (a) Cori, C. F.; Cori, G. T., and Green, A. A.: J. Biol. Chem. **151**:39, 1943. (b) Evans, E. A., Jr., in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 13, p. 187.

phosphate.<sup>35b</sup> Its action is said to be accelerated by reducing substances, such as glutathione, and by a fluoride.<sup>22</sup> Reducing agents act directly on the phosphorylase. The fluoride inhibits alkaline phosphatase and thus blocks dephosphorylation. This permits the accumulation of hexose-phosphoric ester, which would otherwise quickly disappear. The fluoride is thus only an indirect accelerator. Neither our experiments nor any reports discovered in the literature furnish information concerning the effects of the inorganic nephrotoxic agents used by us on this enzyme. Until these effects are known it will not be possible to interpret our results accurately.

The concept that phlorhizin produces its effects on the tubular absorption of dextrose by blocking phosphorylation appears to have originated with Lundsgaard.<sup>25</sup> In 1933 he reported that in digestion experiments with muscle *brei* (broth) and with muscle, yeast and kidney extracts phlorhizin in fiftieth molar to two-hundredth molar concentrations inhibited the processes of esterification and dephosphorylation. He then believed that, because of the absorption of water in the proximal convoluted tubules, a concentration of phlorhizin might be attained in the kidneys capable of inhibiting the phosphorylation of glucose. In later experiments (1935) he concluded<sup>26</sup> that the concentration of phlorhizin in the kidneys necessary to induce maximal action (glycosuria) was less than one fifth of that required to produce the inhibiting effects observed in his earlier experiments. He admitted that from these experiments no support can be gained for the assumption that the action of phlorhizin on the kidneys is due to the earlier demonstrated effect of the poison on esterification *in vitro*. From their own experiments, Walker and Hudson<sup>36</sup> came to a similar conclusion.

Most investigations on the action of phlorhizin have been based on the use of relatively large doses of the glucoside. Lundsgaard<sup>25</sup> used from 36 (two-hundredth molar) mg. to 9.44 (fiftieth molar) mg. Walker and Hudson<sup>36</sup> used doses that were never less than 30 mg. per kilogram, and in the majority of their experiments the dose was from 150 to 550 mg. per kilogram. Throughout the experiments reported in this series of papers we intentionally used very small doses of each of the poisons employed. Our dogs were given 0.5 and 1.0 mg. per hundred cubic centimeters of blood intravenously, which is close to the same amount per kilogram of body weight, and yet these doses produced glycosuria.

36. Walker, A. M., and Hudson, C. L.: Am. J. Physiol. **118**:130, 1937.

Kalckar,<sup>37</sup> however, has described active oxidative phosphorylation in extracts of kidney which was inhibited by phlorhizin. He thus revived Lundsgaard's<sup>25</sup> original hypothesis. Much of the more recent work has followed the course set by Kalckar.

The problem of the relation of phlorhizin to phosphorylation is not as simple as the hypothesis of Wilmer<sup>7</sup> and others suggests. If phlorhizin interferes with the absorption of glucose solely by blocking its phosphorylation, it should not interfere with the absorption of other substances that cannot be phosphorylated or with other functions of the kidneys. This is not the case. Ellinger and Lambrechts,<sup>38</sup> using frogs, found that phlorhizin interfered with reabsorption from the proximal convoluted tubules of rhodamine B. S., and of fluorescein: The former is capable of phosphorylation, while the latter cannot be phosphorylated. White<sup>39</sup> found that phlorhizin lowers the transfer of diodrast as much as 60 per cent. This substance is excreted but is not absorbed by the tubular epithelium. This observation suggests that the effect of phlorhizin on the kidneys is more widespread than that of merely interfering with phosphorylation. Kalckar<sup>40</sup> has stated that this glucoside is not a specific inhibitor of phosphorylation. Lambrechts<sup>41</sup> has also stated that phlorhizin acts not by an inhibition of phosphorylation but by a toxic action on the cells. London and Kotschneff<sup>42</sup> presented evidence that phlorhizin involves organs in addition to the kidneys and causes a toxic disturbance more widespread than glycosuria. Furthermore, Shannon<sup>43</sup> has stated that preliminary experiments on otherwise normal dogs suggest that phlorhizin may inhibit the active tubular reabsorption of glucose by entering into competition with glucose for the transport mechanism and by displacing glucose from it in much the same way in which glucose can exclude xylose.

The most fundamental criticism of the concept that phlorhizin produces glycosuria by blocking phosphorylation has been presented by Weiss-

37. Kalckar, H.: Skandinav. Arch. f. Physiol. **77**: 46, 1937.

38. Ellinger, P., and Lambrechts, A.: J. Physiol. **89**:30P, 1937.

39. White, H. L.: Am. J. Physiol. **130**:582, 1940.

40. Kalckar, H.: Nature, London **136**:872, 1935.

41. Lambrechts, A.: Arch. internat. de physiol. (suppl.) **44**:1, 1937.

42. London, E. S., and Kotschneff, N.: Arch. f. exper. Path. u. Pharmakol. **178**:700, 1935.

43. Shannon, J. A., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1942, vol. 4, p. 297.

berger.<sup>44</sup> In her studies of phosphorylation she used "tagged" radioactive phosphorus. By ordinary analytic methods, only the amounts and the changes in the amounts of compounds in the tissues are observable. By the use of isotopes it is possible to demonstrate both the rates and the changes in the rates at which chemical reactions proceed in the body. Since the alleged inhibition of phosphorylation by phlorhizin need not be accompanied by change in the absolute or the relative amounts of the phosphorus compounds in the tissues concerned but only by change in the rate of phosphorus turnover, radioactive phosphorus furnishes a useful tool for the examination of this theory. A comparison of phosphorus turnover after the administration of radioactive phosphorus revealed no differences in normal and phlorhizined rats in the rate of incorporation of the radioactive phosphorus into the kidney, the intestine, the blood or the liver or into the renal, the hepatic or the intestinal phospholipids. Neither was there any difference in the excretion of radioactive phosphorus in the two groups of rats, although the phlorhizined animals showed marked diuresis and glycosuria. Contrary to the hypothesis that the biologic action of phlorhizin is due to retardation of phosphorylation, Weissberger concluded that phlorhizin does not inhibit processes of phosphorylation in the intact animal and that it must produce its effect by some other mechanism.

Phlorhizin produces glycosuria without altering the amount or the location of the phosphatase, without visibly injuring the cells of the tubules and without disrupting the structural pattern of the kidneys. The inorganic chemical agents used in these experiments produce glycosuria only (1) after markedly altering the distribution, but without necessarily reducing the amount, of phosphatase in the kidneys, (2) after causing extensive necrosis of the tubular epithelium and (3) after completely disrupting the structural pattern of a considerable portion of the proximal convoluted tubules. The mechanism of phlorhizin glycosuria is not, therefore, comparable to that of the glycosuria induced by these inorganic poisons.

44. Weissberger, L. H.: *J. Biol. Chem.* **139**:543, 1941.

Shannon<sup>45</sup> has stated that "there is present in the tubular epithelium of all species a limited amount of a stable cellular element whose specific function it is to combine temporarily with the solute transferred." In the various attempts to explain the glycosuria caused by certain chemical agents, one factor has not been given adequate attention. Each organ performs its functions through the medium of its own peculiar structural pattern. In dogs poisoned with the nephrotoxic substances used in these experiments, not only were the epithelial cells of the tubules killed but the structural pattern of the tubules was disrupted. Under such conditions, although the enzymes or the "stable cellular element" of Shannon,<sup>45</sup> on which a particular process depends, may be present and active in normal, or even in excessive, amounts they cannot function because of alterations in the anatomic structure on which their action depends. This principle would apply whatever may be the mechanism of the reabsorption of sugar by the proximal convoluted tubules.

#### SUMMARY

Glycosuria was produced in dogs with uranyl nitrate, mercury bichloride, potassium dichromate and phlorhizin.

A marked difference was observed in the power of the three inorganic chemical agents to produce glycosuria. Uranyl nitrate was the most potent.

Some degree of correlation was found between the glycosuria and (a) the necrosis of the tubular epithelium, (b) the albuminuria and (c) the chemical agent and its dose.

No definite or constant correlation was observed between the degree of glycosuria and the levels of sugar and urea in the blood or the amount of phosphatase in the blood or in the cortex of the kidney.

Our results shed no light on the possible role of phosphatase in the absorption of glucose by the kidneys because no information is available concerning the effect of the inorganic chemical agents used on phosphorylase.

Certain fundamental differences are presented in the conditions and in the mechanism of the glycosuria induced by phlorhizin and that caused by inorganic poisons.

45. Shannon, J. A.: *Physiol. Rev.* **19**:63, 1939.

## LEUKOPENIA AND INFLAMMATION

The Presence of a Leukopenic Factor in Inflammatory Exudates

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A NUMBER of inflammatory conditions are accompanied by a fall in the number of leukocytes in the blood stream, a so-called state of leukopenia. Lawrence<sup>1</sup> recently discussed the varying conditions which may bring about this picture. These conditions may involve inhibition of the maturation of leukocytes, active elimination of these cells or possibly their destruction. Fitz-Hugh and Krumbhaar<sup>2</sup> have considered agranulocytosis, first described by Schultz,<sup>3</sup> as the result of an arrest of the development of leukocytic elements. In its severe state the disease involves lymphoid cells as well as granulocytes. For this reason it is referred to as pernicious leukopenia.<sup>2</sup> Profound leukopenia referable to a virus infection has been recently reported to occur in cats.<sup>4</sup> It is interesting to note on close scrutiny the frequency with which some infection accompanies an agranulocytic process.<sup>2</sup> Dameshek<sup>5</sup> and Sturgis<sup>6</sup> recently reviewed the literature on the subject of leukopenia. Sturgis<sup>6</sup> expressed the belief that agranulocytic conditions occur frequently as a result of drug sensitizations. He directed attention to such drugs as aminopyrine<sup>7</sup> and gold salts. Sturgis<sup>6</sup> expressed the view that the mechanism is a delay in the maturation of granulocytes in the bone marrow. With reduction in number of leukocytes, susceptibility to infection is increased.<sup>6</sup> Bacterial invasion in various parts of the body results

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1. Lawrence, J. S.: J. A. M. A. **116**:478, 1941.
2. Fitz-Hugh, T., and Krumbhaar, E. B.: Am. J. M. Sc. **183**:104, 1932.
3. Schultz, W.: Deutsche med. Wchnschr. **48**:1495, 1922.
4. Lawrence, J. S.; Syvertson, J. T.; Shaw, J. S., and Smith, F. P.: Am. J. Path. **16**:333, 1940. Hammon, W. D., and Enders, J. F.: J. Exper. Med. **69**:327, 1939.
5. Dameshek, W., in Christian, H. A.: Oxford Medicine, New York, Oxford University Press, 1944, vol. 3.
6. Sturgis, C. C.: Clinics **1**:492, 1942.
7. Sturgis, C. C., and Isaacs, R.: Tr. A. Am. Physicians **46**:328, 1934.

in cellular damage and necrosis, which are characteristically found in agranulocytosis.<sup>6</sup> In my mind it is questionable whether the leukopenia always precedes the infection or whether the severe inflammatory condition per se may not be a predisposing factor in favoring the development of the leukopenic phase. The mechanism of the persistent leukopenia encountered in various inflammatory processes—for instance, typhoid, influenza or tuberculosis—has not been satisfactorily explained.

I have demonstrated the presence of an injurious factor located in, or at least closely associated with, the englobulin fraction of inflammatory exudate.<sup>8</sup> This substance has been termed necrosin. It has been identified not only in canine exudates but also in human exudative material.<sup>8</sup> Smith and Smith<sup>9</sup> have recently confirmed the observation that this substance is present in canine exudates. They<sup>9</sup> have pointed out the presence of a closely similar toxic material in menstrual blood. This is not wholly surprising, for such material contains disintegrated cellular products from the endometrium combined with elements from the blood. Menstrual blood can perhaps be considered as a sort of modified physiologic exudative material, provided exudate, as such, is defined as the products of cellular injury combined with a varying amount of hematic elements. Menstrual blood is therefore viewed by me as primarily a hemorrhagic type of a somewhat modified exudative material. The additional rupture of endometrial vascular structures doubtless is responsible for the hemorrhagic appearance of this exudate. As pointed out in an earlier publication, necrosin shows proteolytic activity which seems to be capable of being held in abeyance by an antiprotease.<sup>10</sup> The observation of this enzymatic property, which may yet prove to be independent of the toxic substance in necrosin, has also been confirmed.<sup>9</sup>

Recent studies indicate the more frequent but not invariable occurrence of necrosin in exudates derived from inflammations at an acid  $p_H$ .<sup>11</sup> It must be pointed out in this connection, however, that necrosin has at times been obtained from exudates at an alkaline  $p_H$ . But the material seems to be more frequently present in acid exudates.<sup>11</sup>

The whole euglobulin fraction of exudate not only induces marked cutaneous injury in the rabbit but causes definite fever and pronounced leukopenia in dogs.<sup>12</sup> Subsequent investigations have revealed that the pyrogenic property of the whole euglobulin fraction is referable not to the thermolabile necrosin but to a different but closely associated thermo-

8. Menkin, V.: Arch. Path. **36**:269, 1943.

9. Smith, O. W., and Smith, G. V. S.: Proc. Soc. Exper. Biol. & Med. **59**:116, 1945.

10. Menkin, V.: Am. J. M. Sc. **208**:290, 1944; Science **100**:337, 1944.

11. Menkin, V.: Federation Proc. **4**:149, 1945.

12. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **54**:184, 1943; footnote 8.

stable substance, termed by me pyrexin.<sup>13</sup> The thermostability of pyrexin, on the one hand, and the unimpaired toxicity of purified necrosin in distilled water, on the other, nullifies the contention that pyrexin is referable to the technical procedure adopted in its preparation.<sup>9</sup>

The present communication indicates that in inflammatory exudates there is a leukopenic factor which is not one of the biologic properties of purified necrosin.<sup>14</sup> It is most often associated with pyrexin; yet it can readily be dissociated, at least to a large extent, from this pyrogenic factor. The presence of such a leukopenic factor in inflammatory exudates in large part explains, perhaps, the leukopenia accompanying numerous inflammatory processes. The leukocytosis-promoting factor (abbreviated as the LPF) present in exudates may well mask the ultimate effect of the leukopenic factor.<sup>15</sup> In brief, the final blood picture accompanying an acute inflammatory process may to a large extent depend on the relative concentration of either the leukocytosis-promoting factor or the leukopenic factor now under discussion, both factors being produced at the site of an acute inflammation.

#### EXPERIMENTAL STUDY

To obtain exudative material, 1.5 cc. of turpentine was injected into the canine chest cavity, as previously described.<sup>15</sup> It was found that the leukopenic factor is more likely to be recovered from exudates that are at an acid pH. For this reason the material was recovered from a cavity with a severe pleural inflammation of several days' duration, with its content at an acid reaction, or else, to hasten the process of acidification, the irritant was reinjected into the pleural cavity. This secondary treatment often tended to induce leukopenia in the circulation besides rendering the animal considerably ill. The leukopenic factor, as a rule, was recovered from such exudative type of material.

The whole exudate in amounts ranging from about 5 to 20 cc. was injected intracardially into dogs, and the leukocyte counts were taken approximately every hour for a period of five to six hours from samples of blood obtained by nicking the ear. Under such circumstances it is usually observed that there is an initial drop in the number of circulating leukocytes during the first two hours after the intravascular injection of the material. This is illustrated in chart 1. After an interval the number of leukocytes rises and definite leukocytosis is then in evidence. This is probably referable primarily to the presence of the leukocytosis-promoting factor in the exudate. The data of the various experiments are assembled in table 1. It is quite clear that within the first two hours after the intravascular injection of exudative material there is an appreciable reduction in the number of circulating leukocytes. From an average basal level of 11,672 white blood cells per cubic millimeter there is an average fall of 3,778 in the first two hours following injections of exudate in otherwise normal dogs.

13. Menkin, V.: Arch. Path. **39**:28, 1945.

14. Menkin, V.: Science, to be published.

15. Menkin, V.: Arch. Path. **30**:363, 1940; Dynamics of Inflammation, New York, The Macmillan Company, 1940.

This appreciable lowering of the number of circulating leukocytes in eight experiments indicates the probable presence of a leukopenic factor in such exudative material.

This leukopenic factor is not present in normal blood serum (table 2 and chart 1). In four experiments in which normal blood serum was introduced into the circulation of dogs, it was noted that the number of circulating leukocytes failed to be altered appreciably within several hours after the injections. These

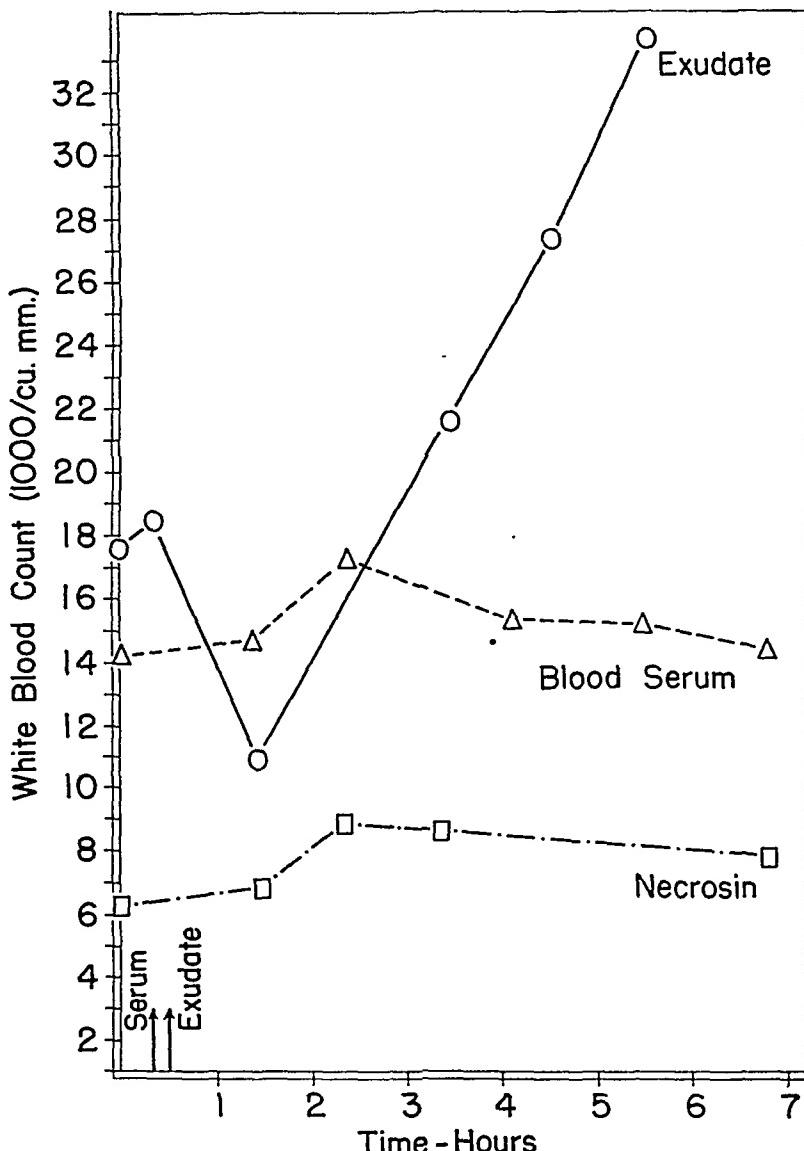


Chart 1.—Effect of necrosin, exudate and serum on the white blood cell count. First, the presence of a leukopenic factor in whole exudate is demonstrated (0—0—0; dog 23-D). The subsequent leukocytosis shown in this curve is likely referable in part at least to the presence of the leukocytosis-promoting factor in the exudative material. That the leukopenic factor is not present in either serum (dog 4-27) or necrosin (dog 29-D) is shown in the two lower curves.

observations clearly indicate that a leukopenic factor is present in exudate but not in normal nonhemolyzed blood serum.

A series of observations was made on dogs on days on which they were not given injections of any material. Some of these animals were the same ones that had been given injections of various fractions of exudates on other days. These experiments were made with the purpose of establishing the normal fluctuation in the white blood cell counts of untreated dogs and also with the view of determining the lowest level to which the white blood cell count usually falls within a period of six to seven hours of study. The data from these experiments are collected in table 3. It is clear that the lowest level of circulating leukocytes is not markedly lower than the initial level. While the average basal level is 10,222 white blood cells per cubic millimeter, the lowest level reached

TABLE 1.—*Evidence That a Leukopenic Factor Is Present in Whole Exudate at an Acid pH*

Dog	White Blood Cell Counts		
	Basal Count	Initial Count Within 1 to 2 Hr. After Intravascular Injec- tion of Exudate	Drop in Count
4-27.....	14,950	9,850	5,100
4-14.....	10,900	7,400	3,500
4-14.....	11,925	8,325	3,600
4-25.....	9,175	3,750	5,425
4-17.....	7,875	7,675	300
18-D.....	9,350	8,300	1,050
18-D.....	10,700	6,950	3,750
23-D.....	18,500	11,000	7,500
Average.....	11,672	7,894	3,778

tuation in the white blood cell counts of untreated dogs and also with the view of determining the lowest level to which the white blood cell count usually falls within a period of six to seven hours of study. The data from these experiments are collected in table 3. It is clear that the lowest level of circulating leukocytes is not markedly lower than the initial level. While the average basal level is 10,222 white blood cells per cubic millimeter, the lowest level reached

TABLE 2.—*Evidence That a Leukopenic Factor Is Not Present in Normal Blood Serum*

Dog	White Blood Cell Counts		
	Basal Cell Count	Lowest Count Within Several Hours of Study	Change in Count
4-14.....	7,575	7,525	- 50
4-27.....	14,150	14,300	+ 150
4-19.....	5,855	5,575	- 280
4-29.....	7,100	7,350	+ 250
Average.....	8,670	8,638	+ 18

within six to seven hours averages 9,225. The difference is therefore clearly not appreciable. As indicated in three experiments, the temperature in such untreated animals likewise scarcely fluctuates (table 3).

These observations indicate that a leukopenic factor is present in exudate and that the evidence is not a matter of normal fluctuation in the number of circulating leukocytes. Furthermore, the factor is more likely to be recovered, though not exclusively, from exudate which is at an acid  $pH$  at the time it is withdrawn from the thoracic cavity. The factor is not present in blood serum, as indicated in the foregoing paragraph.

A search was undertaken in an endeavor to determine whether the leukopenic factor can be isolated from inflammatory exudates. In an earlier study<sup>8</sup> it was

shown that the whole euglobulin fraction of exudate, besides being capable of inducing marked injury of tissue, has pyrogenic and leukopenic properties. Further purification of the euglobulin fraction has indicated that the toxic property is referable to the euglobulin part, and this factor has been termed necrosin, while the pyrogenic effect has been ascribed to another substance associated with the euglobulin fraction and accordingly named pyrexin.<sup>13</sup> Is the leukopenic property of the whole euglobulin fraction<sup>8</sup> associated with necrosin or with pyrexin?

TABLE 3.—*Lowest Level to Which Circulating Leukocytes Fell During a Study of Normal Variations in Their Number in Days*

Dog	White Blood Cell Counts			Change in Temperature, C.		
	Initial Count	Lowest Count During Period of Study (6 to 7 Hr.)	Absolute Drop in Count	Initial Temp.	Highest Temp. During Period of Study	Fall in Temp.
8-D	13,150	12,600	— 550	38.55	38.50	—0.05
18-D	8,000	7,500	— 500	38.65	38.55	—0.10
12-D	6,350	6,500	+ 150	38.75	38.50	—0.25
4-27	10,050	9,150	— 900			
4-23	7,900	6,825	—1,075			
4-19	11,675	10,775	— 900			
4-27	16,500	13,200	—3,300			
7-00	8,150	7,250	— 900			
Average	10,222	9,225	— 997			—0.13

Necrosin when purified further as described previously<sup>13</sup> fails to reduce the number of circulating leukocytes. This is clearly seen in table 4. The introduction of varying amounts of necrosin is utterly unable to decrease the white blood cell count. If anything, there is a slight, probably insignificant, rise in the absolute number of leukocytes (table 4).

TABLE 4.—*Effect of Purified Necrosin on the White Blood Cell Count*

Dog	White Blood Cell Counts		
	Basal Count	Lowest Count Within Several Hours After Injection of Necrosin	Change in Count
6-D.....	10,600	10,600	0
6-D.....	21,350	28,250	+6,950
15-D.....	18,000	15,700	-2,300
28-D.....	5,500	8,000	+2,500
29-D.....	6,250	6,850	+ 600
Average.....	12,340	13,880	+1,540

Pyrexin, on the other hand, not only induces a marked rise in temperature, averaging 1.7 C., but causes a marked reduction of the number of circulating leukocytes in the dog (table 5). Boiling pyrexin in no way alters its pyrogenic and leukopenic properties (table 5). The doses of pyrexin employed ranged from 11 to 66 mg. The average basal leukocytic count was 12,625. The lowest level reached, usually in the first two hours after the injection of pyrexin, averaged 2,645 cells—an average drop of 9,980. A typical experiment is illustrated in chart 2. It is interesting to note that several hours after the injection of pyrexin following the leukopenic phase leukocytosis may develop, (chart 2). The

exact mechanism of this development is not clear, but it is now under investigation.<sup>16</sup> The evidence presented in table 5 indicates that the leukopenic factor in exudate (table 1) is probably closely associated with the pyrogenic factor, pyrexin. None of

TABLE 5.—Effect of Pyrexin on the White Blood Cell Count

Dog	Dose of Pyrexin, Mg.	White Blood Cell Counts			Change in Temperature, C.		
		Basal Count	Lowest Count	Drop in Count	Basal Temp.	Maximal Temp.	Rise in Temp.
8-D	35 (boiled)	11,750	3,450	8,300	38.75	40.45	+1.7
12-D	24 (boiled)	11,650	2,500	9,150	38	40.6	+2.6
18-D	45 (boiled)	11,900	2,050	9,850	38.6	39.95	+1.35
12-D	23	8,650	1,700	6,950	38.55	40.15	+1.6
12-D	29	16,650	2,600	14,050	38.75	41.2	+2.45
8-D	45	10,250	2,750	7,500	38.45	40.0	+1.55
17-D	24	15,600	1,200	14,400	38.95	40.5	+1.55
18-D	11	8,650	4,150	4,500	38.7	39.0	+0.3
18-D	66	10,000	2,150	7,850	38.7	40.55	+1.85
31-D	54	21,150	3,900	17,250	38.05	40.0	+1.95
Average.....		12,625	2,645	9,980			+1.7

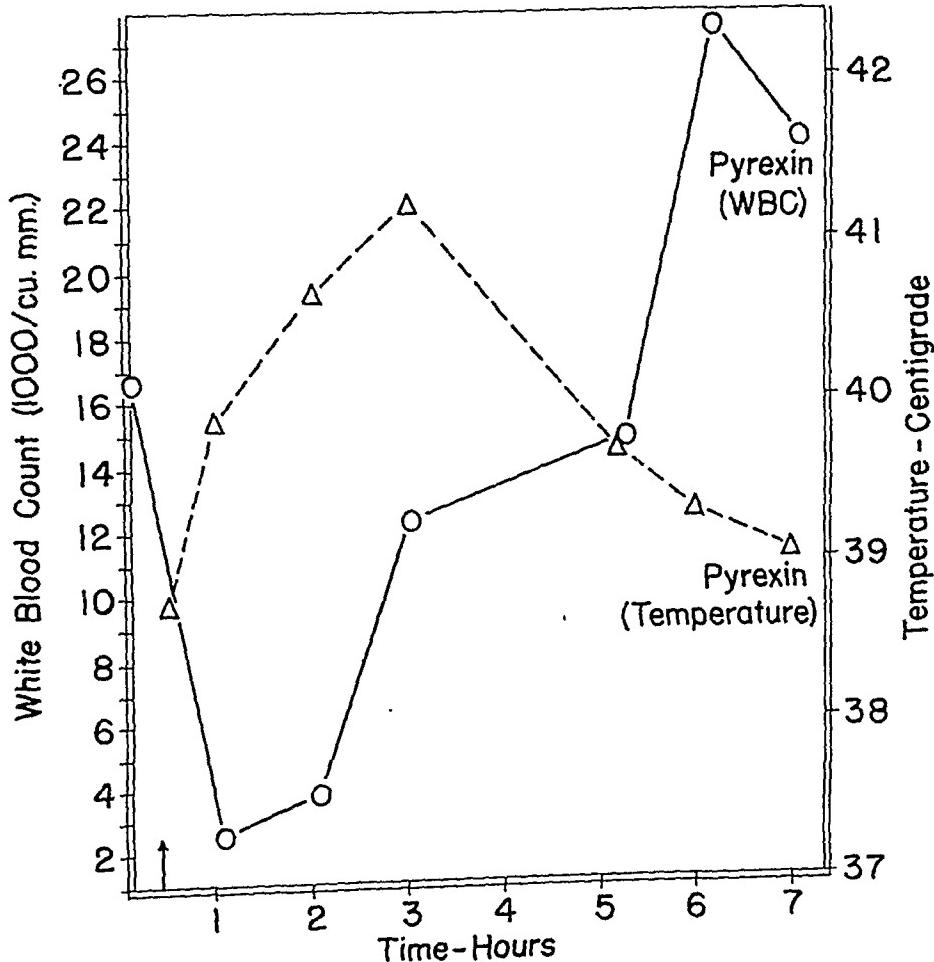


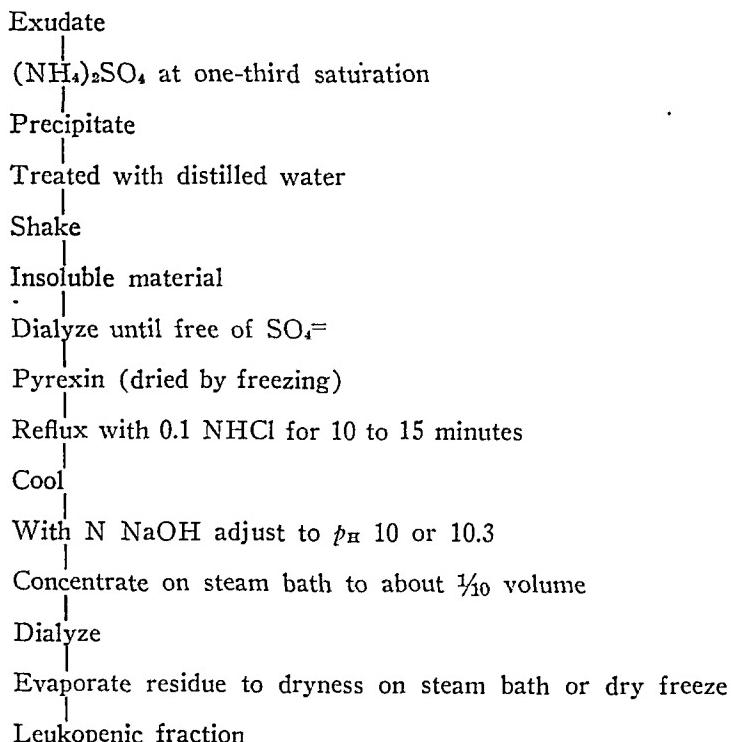
Chart 2.—Effect of pyrexin on the white blood cell count and on the temperature (dog 12-D).

16. The secondary leukocytosis following the injection of pyrexin is perhaps referable to a release into the circulation from various tissues of leukocytes trapped during the leukopenic phase (see footnote at end of this article).

the other fractions studied—for instance, the euglobulin or the pseudoglobulin—induces leukopenia in dogs. The whole exudate and pyrexin, derived from it, seem to be the materials capable of producing consistent leukopenia in dogs.

An attempt was made to determine whether the leukopenic property of pyrexin represents a chemical factor different from the pyrogenic one, or whether it pertains to the same factor as the pyrogenic property. The close association of the two effects may well indicate that one is dealing with a single substance. The fact that pyrexin can be boiled without diminishing its activity<sup>18</sup> and unpublished studies on the amino nitrogen before and after hydrolysis have indicated that one is possibly dealing with a polypeptide, but one which possibly is linked with some prosthetic groupings.<sup>17</sup> It seemed reasonable to me that one may be concerned with a mixture of polypeptides in the partially purified pyrogenic and leukopenic material termed pyrexin. For this reason pyrexin obtained from exudative material as described in an earlier communication<sup>18</sup> was partially hydrolyzed with tenth-normal hydrochloric acid for ten to fifteen minutes. It was observed that a dissociation occurred. By this procedure the pyrogenic property of pyrexin can be essentially eliminated, while the leukopenic activity is left practically intact. The actual scheme of extraction utilized is conveniently arranged in a diagrammatic form:

#### SCHEME OF THE EXTRACTION OF THE LEUKOPENIC FACTOR



After the refluxing of 20 to 25 mg. of pyrexin in 100 cc. of tenth normal hydrochloric acid, the material is cooled and brought to an alkaline  $pH$  with normal sodium hydroxide. After the material has been concentrated, it is dialyzed against tap water to dispose of the excess of hydrochloric acid and sodium hydroxide.

17. A prosthetic group is a non-nitrogenous group linked to either a protein or a protein-derived molecule.

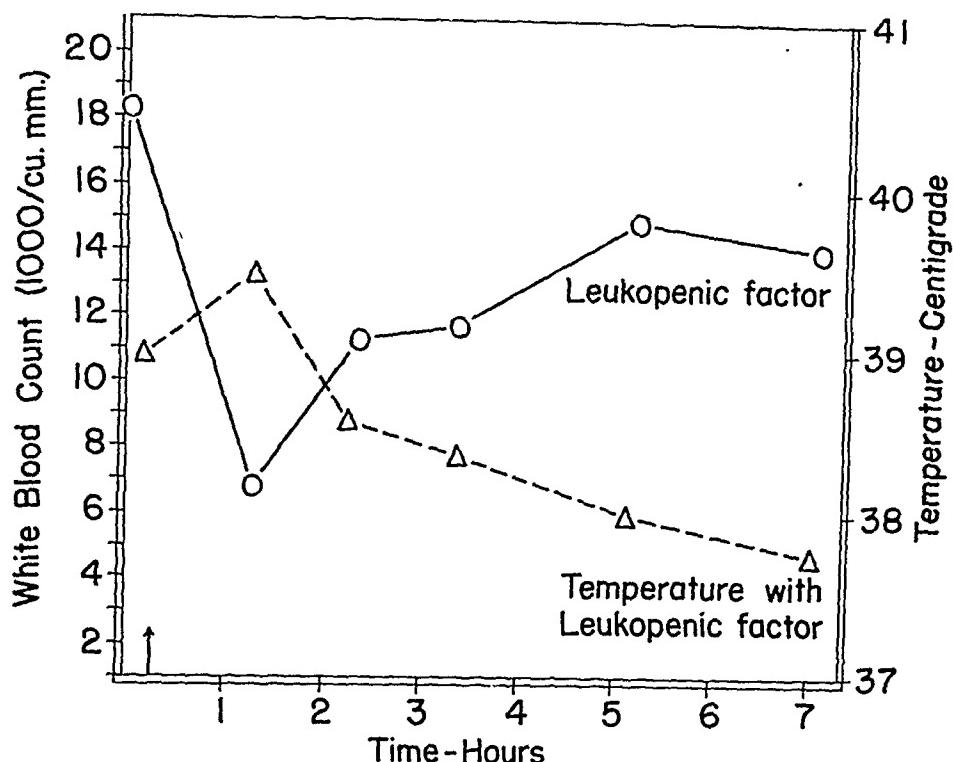


Chart 3.—Dissociation of the leukopenic factor from the pyrogenic factor in pyrexin. The essential dissociation was accomplished by incomplete hydrolysis of pyrexin (dog 26-D). Although the effect of the leukopenic factor is evident, the pyrogenic factor has to a large extent been inactivated.

TABLE 6.—Dissociation of the Leukopenic Factor from the Pyrogenic Factor in Pyrexin

Dog	White Blood Cell Counts			Change in Temperature, O.		
	Basal Count	Lowest Count Within 1 to 6 Hr. After Injection of Material	Drop in Count	Basal Temp.	Maximal Temp.	Change in Temp.
8-D	12,300	7,250	5,050	39.0	38.8	-0.2
18-D	10,250	5,150	5,100	38.45	38.95	+0.5
18-D	10,350	3,900	6,450	38.95	39.55	+0.6
18-D	10,450	4,400	6,050	38.35	38.76	+0.4
8-D	12,400	4,100	8,300	38.75	39.3	+0.55
18-D	12,600	7,350	5,250	38.85	38.9	+0.05
18-D	15,300	7,800	7,500	38.75	38.85	+0.1
18-D*	8,500	4,250	4,250	38.55	39.05	+0.5
31-D*(9 mg.)	22,000	18,600	8,400	38.35	38.5	+0.15
8-D*	10,800	2,950	7,850	38.8	38.8	0
8-D*(2 mg.)	11,250	5,400	5,850	38.8	38.5	-0.3
26-D*	17,800	6,800	11,000	38.95	39.45	+0.5
18-D†	9,200	4,400	4,800	38.55	39	+0.45
18-D†	6,400	4,300	2,100	38.35	38.55	+0.2
18-D‡	5,450	4,300	1,150	38.8	38.25	-0.55
18-D‡	9,900	9,900	0	38.8	38.25	-0.55

\* The leukopenic factor was obtained after dialyzing to remove the inorganic materials used in its preparation. The leukopenic factor was dried and the amount of material injected is indicated.

† The period of hydrolysis in tenth-normal hydrochloric acid was one hour instead of the usual ten to fifteen minutes.

‡ Hydrolysis was carried on for one hour in twice normal hydrochloric acid.

¶ The leukopenic factor was dissociated from the pyrogenic factor by hydrolyzing the latter with 50 per cent hydrochloric acid in an autoclave.

This fraction is now highly leukopenic and is essentially nonpyrogenic. The data concerning the dissociation of the original pyrexin are assembled in table 6. In the first seven experiments the leukopenic factor was obtained without dialyzing to separate the excess inorganic material. Nevertheless the leukopenic factor was as effective as when the excess inorganic material was disposed of by dialysis. This is readily seen by comparing the first seven experiments with the next five experiments on table 6 (marked with a single asterisk). In brief, while the leukopenic activity was maintained, the pyrogenic capacity had become negligible; the two factors had been dissociated from each other. The leukopenic fraction can either be dried on a steam bath or lyophilized in a dry

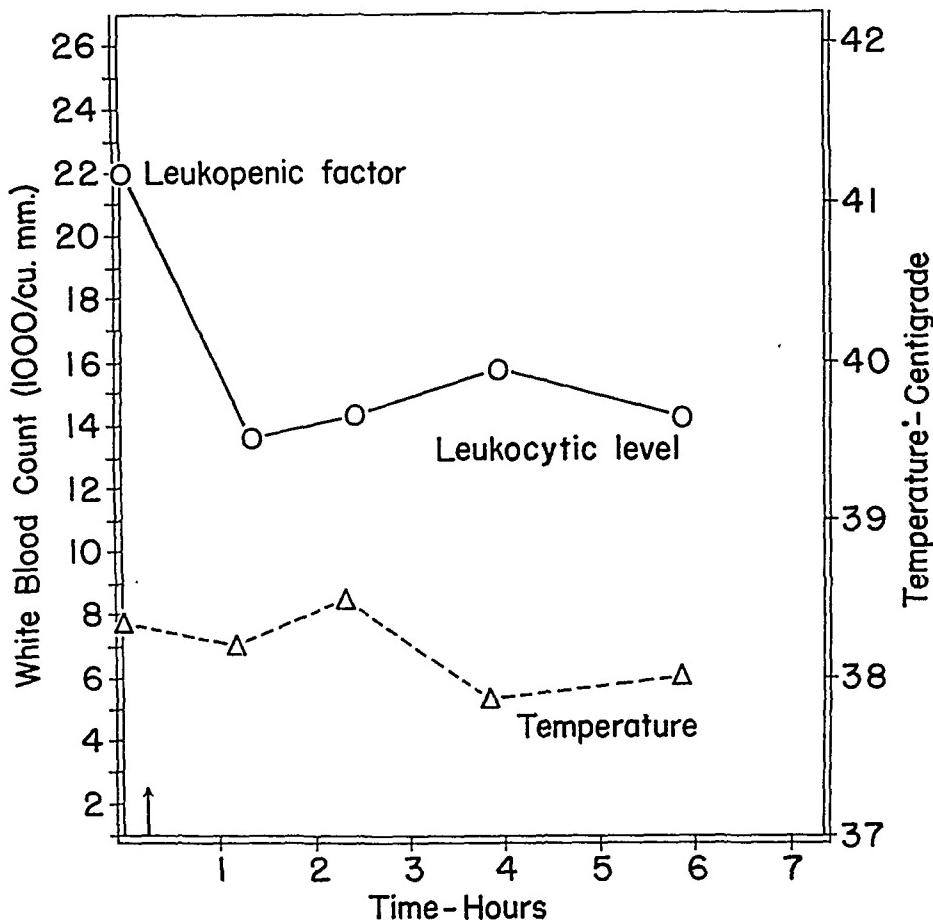


Chart 4.—Dissociation of the leukopenic factor from the pyrogenic factor of pyrexin. The latter factor has been completely inactivated, while the leukopenic factor has been left essentially intact (dog 31-D).

freezing apparatus. When obtained by such a technic, the leukopenic factor of exudate is found to induce leukopenia without being pyrogenic (table 6). The dissociation of pyrexin to yield a potent leukopenic factor while inactivating the pyrogenic property is illustrated in charts 3 and 4.

Control observations have been made by refluxing with tenth-normal hydrochloric acid alone and then rendering the product alkaline with normal sodium hydroxide. These preparations failed to manifest either leukopenic or pyrogenic activity in dogs (table 7).

## SOME PROPERTIES OF THE LEUKOPENIC FACTOR

The leukopenic factor of exudate apparently reduces the number of white blood cells throughout the circulating blood stream, and therefore the effect does not seem to be referable to a redistribution of white cells in the circulation. This was shown to be the case by injecting the leukopenic factor into the blood stream and then taking samples of blood both from a peripheral vessel, that of the ear, and from the heart. Both the peripheral and the systemic blood sample revealed similarly a marked reduction in circulating leukocytes following the injection of the leukopenic factor.<sup>18</sup>

The leukopenic factor affects apparently the granulocytes as well as the mononuclear cells. Differential counts indicate a marked reduction in the numbers of these two types of leukocytes. A more detailed study of the exact effect on the numbers of leukocytes of various forms has not as yet been undertaken.

TABLE 7.—*Control Observations Made by Refluxing with Tenth-Normal Hydrochloric Acid Alone and Then Rendering the Product Alkaline with Normal Sodium Hydroxide*

Dog	White Blood Cell Counts			Change in Temperature, C.		
	Initial Count	Lowest Count	Change in Count	Initial Temp.	Highest Temp.	Change in Temp.
8-D	11,800	13,700	+1,900	38.2	38.55	+0.35
12-D	16,750	13,950	-2,800	38.85	39.35	+0.5
31-D	13,350	10,700	-2,650	38.45	38.75 (2 hr.)	+0.3
18-D	12,150	10,950	-1,200	38.50	38.1	-0.4
Average	13,513	12,325	-1,188			+0.19

In the various experiments four distinct fractions of pyrexin have been utilized. All these have yielded the same type of results as far as the extraction of the leukopenic factor is concerned.

The leukopenic factor is probably a polypeptide, although it is conceivable that there may be as yet some unknown prosthetic group attached to this active polypeptide. When pyrexin is hydrolyzed for a longer interval either with stronger hydrochloric acid (twice normal) or with 50 per cent hydrochloric acid in an autoclave, both the leukopenic and the pyrogenic property are inactivated. This is indicated in table 6 (experiments marked with the symbols ‡ and §§). These experiments indicate that brief hydrolysis with relatively weak hydrochloric acid inactivates only the pyrogenic factor (pyrexin), whereas prolonged hydrolysis with stronger hydrochloric acid inactivates also the leukopenic

18. The effect of the leukopenic factor is evidently not referable to hemodilution. For instance, the number of red corpuscles was hardly altered when 10 mg. of pyrexin was injected into the circulating blood of a dog. Whereas the white cell count dropped from 13,950 to 2,800 in forty-four minutes the red cell count remained essentially constant during a period of more than three hours.

factor (table 6). This suggests that possibly the latter has more peptide linkages than pyrexin. Further chemical studies are necessary, however, and will accordingly be undertaken.

Finally, preliminary determinations of nitrogen and measurements of amino nitrogen before and after hydrolysis, performed by Dr. Frederick Bernheim, indicate that one is quite likely dealing with a polypeptide. Nitrogen determined on the leukopenic factor was found to be 16 per cent. The amino nitrogen before hydrolysis was found in three analysis to average 0.85 per cent, whereas after hydrolysis the average was 5.2 per cent. These results are not inconsistent with the possibility that the leukopenic factor of exudate is a polypeptide.

#### COMMENT

The observations indicate that the pyrogenic substance, pyrexin, in its present state of purification contains at least two properties, which seem to be closely associated with each other in that particular material. The two properties can be dissociated by subjecting pyrexin to incomplete hydrolysis for a short interval in relatively weak hydrochloric acid. By such a procedure the pyrogenic factor can be inactivated while the leukopenic factor is left essentially intact. Nevertheless, since this process involves the obliteration of the pyrogenic property, it is not yet known whether the leukopenic and the pyrogenic capacities are two properties of the same substance or whether each represents a separate substance in the as yet relatively impure pyrexin. Further studies on the purification of pyrexin and the eventual recovery of separate material entities with their respective pyrogenic and leukopenic properties can definitely establish the concept of two separate factors in the same substance or that of two different substances. Such studies are now in progress.

The finding of a leukopenic factor in inflammatory exudates, particularly at an acid  $p_H$ , is of significance. It may help in further understanding of the leukopenic disturbance in various clinical disorders, such as typhoid, tuberculosis and influenza, as well as in many other pathologic conditions. It is conceivable that the constant production of this leukopenic factor at the site of severe cellular injury may yield a continuous state of leukopenia in the circulation. It is perfectly possible that the ultimate cytologic picture of either leukopenia or leukocytosis in the blood stream with inflammation may depend on whether the release of the leukocytosis-promoting factor or that of the leukopenic factor predominates at the site of injury.

Finally, it has been pointed out in an earlier study<sup>19</sup> that the fundamental stereopattern of inflammation is largely referable to various

19. Footnotes 8 and 13.

common denominators liberated from cells previously injured by an irritant. When these basic substances have reached a sufficient concentration in the injured cells and have been liberated, their effect becomes evident. They include leukotaxine the leukocytosis-promoting factor, necrosin, pyrexin, dextrose<sup>20</sup> and, now may finally be added, the leukopenic factor. It is the liberation of these common denominators which determines the final picture of inflammation. In brief, this concept refers the problem of inflammation to a biochemical study of the injured cell.

#### CONCLUSION

A leukopenic factor is present in inflammatory exudates of dogs, particularly if the exudate gives an acid reaction. When injected into the blood stream of a normal animal, the leukopenic factor causes a rapid fall in the number of circulating leukocytes. In its present state of purification it is found as a rule to be closely associated with pyrexin, the substance primarily concerned in the development of the fever associated with inflammation. It can be dissociated from the pyrogenic factor by subjecting pyrexin to incomplete hydrolysis, which inactivates the pyrogenic factor. The evidence on hand does not as yet prove conclusively that the leukopenic factor is a substance separate from pyrexin. It may be only a separate factor in pyrexin. Further studies are in progress to establish this point. Preliminary observations indicate the probable polypeptide nature of the leukopenic factor.<sup>21</sup>

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20. Am. J. Physiol. **134**:517, 1941; **138**:396, 1943.

21. In subsequent studies, made since this communication was sent to the ARCHIVES for publication, it has been found that, although the leukopenic factor of inflammatory exudate is mostly found in close association with pyrexin, yet it can at times be recovered to some extent in other fractions of exudative material, indicating that it is apparently not exclusively found in association with pyrexin. Furthermore, additional studies seem to indicate that the leukopenic factor of exudates does not primarily deplete the bone marrow, but rather the mechanism involved appears to be a rapid trapping of leukocytes in the alveolar walls of the lungs, in the sinusoids of the liver and apparently in the spleen. The latter fact may be of significance in the further understanding of the mechanism involved in the acute splenic tumor accompanying numerous inflammatory processes.

## Case Reports

### OBSTRUCTION OF THE AORTIC Isthmus BY A CALCIFIED THROMBUS

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OBSTRUCTION of the thoracic portion of the aorta is uncommon, and in the majority of instances it is due to coarctation.<sup>1</sup> Fresh thrombi in this location have been described, but such a thrombus does not occlude the vessel to any pronounced degree.<sup>2</sup> The only example of significant narrowing of the thoracic aorta by an organizing thrombus was reported by Aubertin.<sup>3</sup> An instance of almost complete stenosis of the aortic isthmus by a calcified thrombus is presented in the following communication.

#### REPORT OF A CASE

A 40 year old Negro sandblaster was found to have a positive serologic test for syphilis at an army induction center in February 1943. He received thirty-three injections of a bismuth preparation and six injections of oxophenarsine hydrochloride at irregular intervals during the following ten months.

Initial symptoms in August 1943 were mild substernal discomfort and numbness and tingling in the arms on exertion. Two months later there were additional symptoms of suffocation, palpitation and weakness on exertion. At this time the systolic blood pressure was 200 mm. of mercury. In January 1944, after a particularly severe attack of the previously noted symptoms, the patient came to the New Haven Dispensary. At no time did he suffer from dyspnea, orthopnea, edema, headaches or epistaxis. He had continued to perform heavy manual labor.

He was an extremely well developed, well nourished, middle-aged Negro, who appeared in no acute distress. The fundi showed sharply outlined disks and slightly narrowed, tortuous vessels. The lungs were clear to percussion and auscultation. The forceful point of the maximal impulse of the heart was localized in the fifth interspace in the anterior axillary line. No thrills were felt. The sounds were of good quality, with the aortic greater than the pulmonic second sound. The rate was 66 per minute; the rhythm, regular. There was a harsh systolic apical murmur, transmitted to the axilla, as well as a loud harsh systolic murmur in the aortic region, transmitted to the cervical vessels on the right side. The blood pressure was 185 systolic and 70 mm. diastolic. The liver was palpable

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From the Department of Pathology, Yale University School of Medicine.

1. Hamilton, W. F., and Abbott, M. E. Am. Heart J. **3**:381 and 574, 1928. Lewis, T.: Heart **14**:205, 1933.

2. Welch, W.: Embolism and Thrombosis, in Allbutt, T. C., and Rolleston, H. D.: System of Medicine, New York, The Macmillan Company, 1909, vol. 15. Desclin, L.: Frankfurt. Ztschr. f. Path. **40**:520, 1930. Hancock, J. C.: J. Iowa M. Soc. **31**:543, 1941.

3. Aubertin, M. C.: Ann. de méd. **10**:454, 1921.

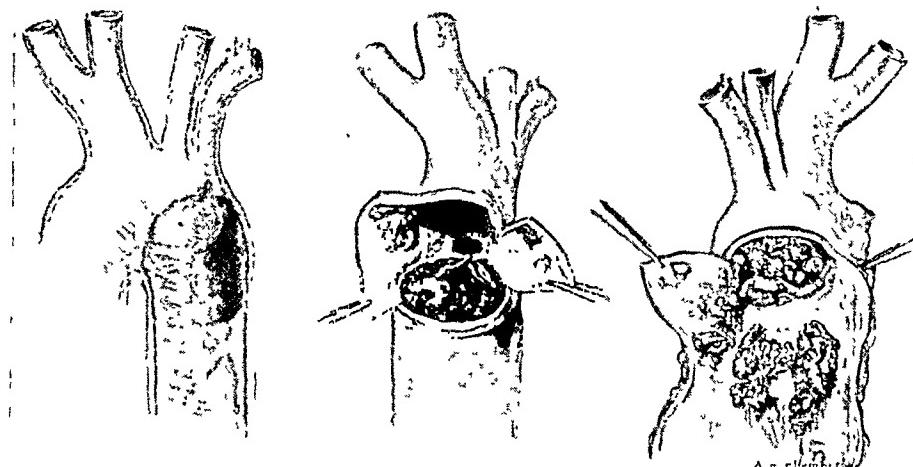
2 fingerbreadths below the costal margin. No edema of the extremities was noted, and the pulsations in the dorsalis pedis artery were of good quality. Otherwise the examinations, including that of the urine, showed no marked deviations from the normal.

The clinical diagnosis was cardiovascular syphilis with syphilitic aortitis, coronary insufficiency, dilatation of the aorta, left-sided cardiac hypertrophy and hypertension.

The patient was subsequently followed in the dispensary, where a roentgen examination revealed cardiac enlargement and calcification of the aortic knob. Treatment with glyceryl trinitrate afforded great relief. Arteriosclerotic heart disease and calcific aortic stenosis were added to the diagnosis.

On Nov. 16, 1944, the patient reached the emergency room of the New Haven Hospital coughing up frothy, blood-tinged sputum. Treatment for acute pulmonary edema with theophylline ethylenediamine, morphine and oxygen did not prevent a prompt fatal outcome.

*Pertinent Anatomic Findings.*—The body was muscular and well developed. There was bilateral hydrothorax. The heart weighed 600 Gm., and the left



Drawings of the aorta with obstruction at the isthmus.

ventricular muscle measured 17 mm. in thickness. The free edges of the cusps of the aortic valve were slightly thickened, and the commissures were only 2 mm. wide. The aortic valve measured 7.5 cm. in circumference and did not appear insufficient. The ostium of the left coronary artery was narrowed to 2 mm. in diameter.

The aorta was occluded for a distance of 3 cm. beyond the mouth of the subclavian artery by a stony-hard, irregularly shaped mass which, as seen on cross section, left a crescent-shaped lumen measuring 3 mm. at the widest point (figure). The decalcified microscopic preparation showed the occluding material to consist of densely packed fibrin with organization at the periphery and small zones of bone formation. The elastic media at this level was interrupted by vascular scars. The adventitia consisted of a thick layer of acellular, dense fibrous connective tissue, and there were accumulations of plasma cells and lymphocytes around the vasa vasorum. Similar syphilitic lesions, as well as intimal fibrosis and atheromatosis, were present in other portions of the thoracic aorta. Smaller mural calcified thrombi occurred above and below the obstruction. The internal mammary, intercostal and long thoracic arteries were not enlarged.

COMMENT

The nature of the aortic lesion indicated that obstruction had been present for a considerable period. It is not uncommon for extreme stenosis of the aortic valve to exist for a long time with only slight circulatory embarrassment. In such circumstances there is no opportunity for the development of collateral circulation. These observations may explain the fact that the narrow lumen of the aorta in this patient allowed adequate circulation until failure of the left ventricle occurred.

SUMMARY

A calcified thrombus was observed in a patient with cardiovascular syphilis which produced almost complete obstruction of the isthmus of the aorta.

## General Reviews

### TROPICAL DISEASES

#### Involvement of the Nervous System

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WITHIN recent years, owing to the shifting of large numbers of the population to many points throughout the world, particularly to the subtropics and the tropics, a great deal of interest has been manifested in the various diseases most characteristic of these regions. As a result, many reports and publications have appeared dealing with the subject of the tropical diseases. Curiously enough, most of the recent literature, even when exhaustive, has ignored almost completely the possible and often probable involvement of the nervous system. It has been recognized that certain of these diseases, such as the rickettsial infections and malaria, produce definite encephalitis, but the fact is overlooked that almost every tropical disease may involve the nervous system to produce encephalic changes with possible residual damage of the brain. Such complications can well be of utmost importance in the final evaluation of the recovery of the patients, especially when large proportions of the population are being exposed and infected. It is for the purpose of emphasizing the encephalic complications of the tropical diseases that the present review was undertaken. Since many excellent reports have already been published describing the general characteristics of these diseases, no attempt will be made in the present review to repeat the data contained in those reports. Only such features will be included as appear necessary to introduce the encephalic complications. In attempting such a study it was felt to be most convenient to group the various diseases according to the natures of the causative organisms.

#### FILTRABLE VIRUSES

*Yellow Fever.*—Yellow fever is an acute infectious type of jaundice caused by a virus which is transmitted from man to man by the mosquito *Aedes aegypti*. It occurs in endemic form as jungle yellow fever in vast areas of tropical South America and Africa, and in epidemic form in various urban communities in equatorial Africa and tropical South and Central America. The onset of the disease is usually sudden, with slight

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chills and fever, headache, pains of the muscles, nervousness and anxiety. These symptoms are followed in a day or two by albuminuria, oliguria, increasing fever, nausea, vomiting and severe prostration. Jaundice appears on the fourth or the fifth day, accompanied by bleeding from the gums and hemorrhages of the stomach and the intestine. Death usually occurs from the sixth to the ninth day, preceded by delirium, prostration and coma. The mortality rates in epidemics have been reported as from 5 to 10 per cent up to 80 per cent.

The essential lesions of yellow fever are found in the liver and are characterized by midzonal necrosis, fatty degeneration and the presence of "councilman bodies." Less striking changes are noted in the spleen, the kidneys, the heart, the lungs and the gastrointestinal tract. Although the virus of yellow fever is highly neurotropic, there have been but few reports dealing with the pathologic involvement of the nervous system. Most investigators have examined the brain superficially and have stated that there were no significant changes (Noguchi<sup>1</sup>; Mackenzie<sup>2</sup>). Schmidt<sup>3</sup> in 1879 expressed the opinion that some patients with yellow fever died from "congestion of the brain." He found fatty degeneration and fatty infiltration of the cortical neurons as well as vascular congestion. Similar observations were reported by Pothier<sup>4</sup> in 1905-1906.

Jacob, Fialho and Villela<sup>5</sup> in the first careful study of the brain in yellow fever found definite encephalitis in 14 cases. They observed mild meningeal infiltration with lymphocytes and macrophages and marked fatty degeneration of the ganglion cells in the cortex, the basal nuclei, the hippocampus and the dentate nucleus. Similar fatty degeneration was found in the endothelial cells of the walls of blood vessels. Many of the neurons were swollen and showed chromatolysis, while others were pyknotic and fragmented. Demyelination and gliosis were noted about many of the blood vessels. In 4 cases perivascular granulomas were found within the gray matter of the medulla and the pons; they were described as focal collections of glial cells intermixed with lymphocytes and surrounded by diffuse glial proliferation. In 6 cases there were marked degeneration and focal changes in the cerebellar cortex. These changes varied from moderate disappearance of Purkinje cells to severe diffuse gliosis with perivascular demyelination.

Stevenson<sup>6</sup> studied the brains of 20 persons who died of yellow fever. He found perivascular hemorrhages in all, most numerous in the

1. Noguchi, H.: *J. Exper. Med.* **29**:547, 1919.

2. Mackenzie, I.: *J. Trop. Med.* **30**:218, 1927.

3. Schmidt, H. D.: *M. Rec.* **16**:44, 1879.

4. Pothier, O. L.: *New Orleans M. & S. J.* **58**:394, 1905-1906.

5. Jacob, A.; Fialho, A., and Villela, E. L.: *Deutsche Ztschr. f. Nervenh.* **111**:111, 1929.

6. Stevenson, L. S.: *Arch. Path.* **27**:249, 1939.

subthalamic and periventricular regions at the level of the mamillary bodies. Slight perivascular edema was noted in some cases and perivascular lymphocytes in others. In only 1 case did he note any loss of nerve cells. Reactive changes in the microglia and the astroglia were slight.

Nicolau, Mathis and Baffet<sup>7</sup> in a case report described marked glial proliferation forming perivascular "cuffing" within the brain substance, with accompanying mononuclear infiltration. Both nerve cells and glial elements contained characteristic oxyphilic inclusion bodies of a type described by them in a previous paper.<sup>8</sup>

In 1928 Stokes, Bauer and Hudson<sup>9</sup> discovered a susceptible laboratory animal, and in 1930 Theiler<sup>10</sup> found that the virus of yellow fever is infectious for the brain of the mouse. These discoveries marked a new era in research on yellow fever. Numerous pathologic studies have been made of encephalitis in monkeys and mice experimentally infected with the virus of yellow fever. Aside from the original study by Hudson and his co-workers,<sup>9</sup> in which no abnormal changes were observed, well marked encephalitic changes have been described. Goodpasture,<sup>11</sup> using monkeys, found extensive acute degeneration and necrosis of ganglion cells. Some of the nuclei of these cells contained coarse acidophilic inclusions. Polymorphonuclear leukocytes diffusely infiltrated the cortical tissues, and there was a thin perivascular mononuclear infiltration. Petechiae were numerous. Lloyd and Penna<sup>12</sup> noted degenerative changes in the nerve cells of the cortex, brain stem and spinal cord, with acidophilic particles in the cell nuclei. There was definite perivascular lymphocytic infiltration of the brain and the spinal cord. Accompanying these changes was a considerable amount of interstitial glial cell infiltration. Findlay and Stern<sup>13</sup> observed the following changes: generalized microglial proliferation, nerve cell changes, which in the monkey consisted of degeneration and neuronophagia, and the presence of a perivascular inflammatory exudate consisting of lymphocytes.

It is apparent from these reports that well defined encephalitis occurs in both man and experimental animal in yellow fever. Particularly in the experimental animal does this virus show highly neurotropic properties.

7. Nicolau, S.; Mathis, M., and Baffet, O.: Bull. Soc. path. exot. **30**:615, 1937.

8. Nicolau, S.; Kopciowska, L., and Mathis, M.: Ann. Inst. Pasteur **53**: 455, 1934.

9. Stokes, A.; Bauer, J. H., and Hudson, N. P.: Am. J. Trop. Med. **8**:103, 1928.

10. Theiler, M.: Ann. Trop. Med. **24**:249, 1930.

11. Goodpasture, E. W.: Am. J. Path. **8**:137, 1932.

12. Lloyd, W., and Penna, H. A.: Am. J. Trop. Med. **13**:1, 1933.

13. Findlay, G. M., and Stern, R. O.: J. Path. & Bact. **40**:311, 1935.

*Dengue.*—Dengue is a common virus disease occurring in both epidemic and endemic form in subtropical and tropical localities. Under favorable climatic conditions, such as warm, wet weather, epidemics of the infection may reach as far north as Philadelphia. The disease was first described in Cairo, Egypt, and Batavia, Java, in 1779 (Beylon<sup>14</sup>) and in Philadelphia in 1780 (Griffetts and Griffetts<sup>15</sup>). There have been nine epidemics in the United States, the last occurring in the Southern states in 1922-1923, at which time 29,827 cases were reported in Louisiana (Scott<sup>16</sup>) and 82,681 cases in Florida (Richardson<sup>17</sup>). The virus is spread chiefly by the mosquito *Aedes aegypti*, although *Aedes albopictus* (the most common oriental mosquito) can also transmit it.

The clinical picture in dengue can be kaleidoscopic, producing disturbances of the gastrointestinal tract, the heart, the adrenal glands and the nervous system, any one of which may dominate the picture (King<sup>18</sup>). The two most characteristic features, however, are the paroxysms of biphasic temperature and the severe pain occurring in the muscles and the joints. Because of these features the disease is occasionally referred to as "breakbone fever," "saddle back fever" and "three day fever." The onset is generally acute, resulting in a high temperature, chills, severe prostration and occasionally a cutaneous rash. In spite of the severe morbidity, most patients recover, and therefore pathologic studies of this illness are few. However, even clinically it is apparent that the nervous system is frequently and often severely implicated. As a matter of fact, some investigators, particularly Apostolopoulos<sup>19</sup> and Pamboukis,<sup>20</sup> expressed the belief that the virus of dengue is chiefly neurotropic and that the clinical manifestations result indirectly from the action of the virus on the nervous system. Aside from the headaches, complaints referable to all parts of the nervous system have been described, such as photophobia, lethargy, paresis, convulsions and even psychic disturbances (Gill<sup>21</sup>; Ghiannoulatos<sup>22</sup>; Richardson<sup>17</sup>; Faver<sup>23</sup>; Bargy<sup>24</sup>).

Studies on the nervous system are not too common, because of the low mortality rate. Catsaras<sup>25</sup> reported cerebral changes in 3 cases of

14. Beylon, D., in Strong,<sup>135</sup> vol. 2.
15. Griffetts, T. H. D., and Griffetts, J. J.: Pub. Health Rep. **46**:2725, 1931.
16. Scott, L. C.: J. A. M. A. **80**:387, 1923.
17. Richardson, S.: Tr. Am. Ophth. Soc. **31**:450, 1933.
18. King, W. W.: New Orleans M. & S. J. **69**:564, 1916-1917.
19. Apostolopoulos, K. G.: München. med. Wchnschr. **77**:265, 1930.
20. Pamboukis, G.: Schweiz. Arch. f. Neurol. u. Psychiat. **26**:51, 1930.
21. Gill, W. D.: Arch. Ophth. **57**:628, 1928.
22. Ghiannoulatos, G. P.: Rev. neurol. **38**:599, 1931.
23. Faver, M.: J. Florida M. A. **24**:395, 1938.
24. Bargy, M.: Bull. et mém. Soc. franç. d'opht. **42**:293, 1929.
25. Catsaras, J.: Arch. f. Schiffs- u. Tropen-Hyg. **35**:278, 1931.

dengue. Histologically, the cortical neurons were swollen and irregular in contour. They often consisted of irregular masses without nuclei. No inflammatory changes were observed. Melissinos<sup>26</sup> reported 3 cases in which the patients presented convulsions, lethargy and coma. The histologic changes were consistent in all cases and were divided by the author into three types, namely: degenerative, inflammatory and hemorrhagic. The degenerative changes consisted of neuronal alterations, evidenced by swelling and chromatolysis. Hemorrhagic foci were observed, scattered throughout the brain. In many of these foci the vessels showed early inflammatory changes with swelling of the vascular endothelium and beginning extravasation of red cells. Inflammatory changes were present independently of the hemorrhagic foci and consisted of vascular hyperemia, perivascular leukocytic infiltration, glial reactions and endothelial proliferation. Numerous small cellular collections were scattered throughout the tissues. These were composed of both leukocytes and glial elements. No areas of softening or demyelination were observed.

#### RICKETTSIAL DISEASES

The rickettsias are minute bacteria-like micro-organisms which live and multiply intracellularly in the tissues of arthropod or animal hosts. The rickettsias which cause diseases of man may be divided into four groups on the basis of epidemiologic, pathologic and immunologic studies. They are: the typhus group, which are transmitted by body lice and fleas; the Rocky Mountain spotted fever group, transmitted by ticks; the tsutsugamushi fever group, transmitted by mites, and the Q fever group, which may be transmitted either by ticks or by droplets from infected persons.

With the exception of Q fever, the rickettsial diseases are clinically, pathologically and immunologically quite similar. They are characterized by sudden onset, rash, fever of fairly well defined duration, various nervous and mental disturbances and prostration. The rickettsias appear to have definite neurotropic tendencies, invading the brain to produce a primary encephalitic process. The principal pathologic changes are proliferative and inflammatory changes in the smaller branches of the vascular system.

*Epidemic and Endemic Typhus.*—Epidemic typhus is an acute infectious disease caused by Rickettsia prowazeki and transmitted from man to man by the louse (*Pediculus humanus*). It is worldwide in distribution, and severe epidemics are associated with war, overcrowding and poor hygienic conditions. Endemic foci of this disease are present in the highlands of Central and South America, North Africa and parts of

26. Melissinos, J.: Arch. f. Schiffs- u. Tropen-Hyg. 41:321, 1937.

Central and South Africa, in southern and eastern Europe and in Asia Minor, India and China.

There is no question that the nervous system manifestations of typhus fever form an important part of the symptom complex (Gerhard<sup>27</sup>; Murchison<sup>28</sup>; Hampeln<sup>29</sup>; Curschmann<sup>30</sup>; Devaux<sup>31</sup>; Rabinovitch<sup>32</sup>; Baeyer<sup>33</sup>; Skliar<sup>34</sup>; Munk<sup>35</sup>; Stockert<sup>36</sup>). Devaux in commenting on the epidemic in Rumania in 1919 stated, "There are few patients who do not present either during the febrile period or during convalescence some more or less grave and persistent trouble indicating central nervous system and peripheral nerve involvement." He described the neurologic complications as consisting during the first week of delirium, convulsions, monoplegias and hemiplegias. During the second week the symptoms showed bulbar localization of the infection. Most neurologic symptoms occurred during convalescence. He reported 215 cases.

Pathologic changes in the central nervous system in typhus were first described by Popoff,<sup>37</sup> in 1875, and by Ivanovskii,<sup>38</sup> in 1876. They both described widespread nodular collections of wandering cells in the pericellular and perivascular spaces of the cerebral cortex, accompanied by an interstitial inflammatory reaction. They observed an increase of nodular elements in the region of the ganglion cells with atrophy and degeneration of the latter.

It was not until 1914 that further studies of the central nervous system in typhus were reported. In that year Alphejewski<sup>39</sup> reported a case in which he found round cell infiltration of the meninges and brain tissue as well as proliferation of cells within the walls of blood vessels, producing obliteration of the lumens, and formation of granulomas resembling miliary gummas. Many of the nerve cells showed swelling, chromatolysis and neuronophagia. Scattered areas of demyelination were visible throughout the cerebral cortex. Prowazek<sup>40</sup> in 1915 studied in particular the structure of the cerebral nodules in this disease and believed that

27. Gerhard, W. W.: Am. J. M. Sc. **20**:289, 1837.

28. Murchison, C.: A Treatise on the Continued Fevers of Great Britain, London, Longmans, Green & Company, 1873.

29. Hampeln, P.: Deutsches Arch. f. klin. Med. **26**:238, 1880.

30. Curschmann, H.; in Nothnagel, H.: Encyclopedia of Practical Medicine, Philadelphia, W. B. Saunders and Company, 1901, vol. 1, p. 475.

31. Devaux, A.: Lancet **1**:567, 1919.

32. Rabinovitch, J. S.: Ztschr. f. d. ges. Neurol. u. Psychiat. **115**:34, 1928.

33. von Baeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **175**:225, 1942.

34. Skliar, N.: Monatschr. f. Psychiat. u. Neurol. **52**:21, 1922.

35. Munk, F.: Med. Klin. **36**:452, 1940.

36. Stockert, F. G.: Deutsche med. Wchnschr. **69**:506, 1944.

37. Popoff, L.: Zentralbl. f. d. med. Wissenschaft. **13**:596, 1875.

38. Ivanovskii, P.: J. dlya normal. i patol. histologii **10**:93, 1876.

39. Alphejewski, N.: Sovrem. psikiat. **8**:279, 1914.

40. von Prowazek, S.: Beitr. z. klin. Infektionskr. **4**:5, 1915-1916.

they were composed of polymorphonuclear leukocytes, isolated endothelial cells, plasma cells and rarely glial elements. Benda,<sup>41</sup> on the other hand, held that these nodules were composed chiefly of glial cells. He noted that the individual nodule was not particularly localized to a vessel and the immediately adjacent tissue; the nodules were present throughout the brain. Ceelen<sup>42</sup> described the formation of the nodule as beginning with proliferation and swelling of the vascular endothelium, associated with proliferation of the adventitial cells and perivascular collection of lymphocytes. He emphasized in particular that the neuroglia participate in the formation of the nodule, that the nodule is constantly associated with a vessel and that there are severe ganglion cell and myelin changes in the vicinity of nodules.

Following these reports, a great number of articles appeared describing the changes in the nervous system in typhus. Many of these are summarized in the works of Wolbach, Todd and Palfrey,<sup>43</sup> Ceelen,<sup>44</sup> Dawydowskie<sup>45</sup> and Hirschberg.<sup>46</sup>

Wolbach, Todd and Palfrey<sup>43</sup> examined 39 cases of epidemic typhus in Poland in 1919-1920 and gave an excellent description of the pathologic alterations of the nervous system. Macroscopically most of the brains appeared normal, but a few showed injection of the meningeal and cortical vessels and petechiae in the basal ganglion, the pons and the midbrain.

The microscopic process was characterized by presence throughout the central nervous system of tubercle-like nodules in some way associated with small vessels. These nodules were most frequent in the medulla, the pons, the midbrain, the basal ganglia and the cerebral cortex, especially the parietal region. The nodules consisted chiefly of neuroglia cells with some polymorphonuclear leukocytes, plasma cells, occasional red cells and rarely endothelial cells. The authors felt that macrophages were important constituents of the nodules.

According to these authors, the earliest lesions of typhus are found in the capillary and precapillary endothelium, which becomes swollen and proliferated, often obliterating the lumen of the vessel. Erythrocytes frequently escape into the perivascular spaces, producing tiny hemorrhages about the vessels. Following this, a mononuclear infiltration

41. Benda, C.: *Ztschr. f. ärztl. Fortbild.* **12**:464, 1915.

42. Ceelen, W.: *Ergebn. d. allg. Path. u. path. Anat.* **19**:307, 1919.

43. Wolbach, S. B.; Todd, J. L., and Palfrey, F. W.: *The Etiology and Pathology of Typhus*, Cambridge, Mass., Harvard University Press, 1922.

44. Ceelen, W.: *Klin. Wchnschr.* **53**:530, 1916.

45. Dawydowskie, J. W.: *Ergebn. d. allg. Path. u. path. Anat.* (pt. 2) **20**:571, 1923-1924.

46. Hirschberg, N.: *Fleckfieber und Nervensystem*, in *Abhandlungen aus der Neurologie, Psychiatrie, Psychologie und ihren Grenzgebieten*, 1932, no. 66.

appears around the affected vessels. There is also a simultaneous reaction of the neuroglia resulting in the formation of small nodules. Polymorphonuclears are invariably present in small numbers, as are also plasma cells and a few red cells. These various lesions were interpreted as representing a definite proliferative reaction preceded by injury of and proliferation of the vascular endothelium. The authors concluded that the endothelial and the neuroglial proliferation were in direct response to the parasite of typhus carried into the nerve tissue by the migration of endothelial cells. Rickettsias were seen and described in the endothelial cells. Ganglion cell changes evidenced by chromatolysis were observed in the nuclei of the medulla and the midbrain and in the Purkinje cells of the cerebellum.

Pathologic changes similar to those reported by Wolbach, Todd and Palfrey had already been noted by a number of investigators. Fraenkel,<sup>47</sup> Aschoff<sup>48</sup> and Jarisch<sup>49</sup> had observed the nodules in the brain. Albrecht<sup>50</sup> stressed the vascular origin of the nodules and noted marked perivascular changes. Herzog,<sup>51</sup> Abrikossoff,<sup>52</sup> Jarisch<sup>49</sup> and Krinitzki<sup>53</sup> expressed the opinion that the vascular changes were due to thrombosis and that the nodules were primarily vascular in origin, secondary to a reparative process. Spielmeyer,<sup>54</sup> on the other hand, suggested that the nodules arose independently of any vascular pathologic process. Lisen<sup>55</sup> also held that the glial reaction was more sensitive in typhus than the reaction of the mesodermal elements. He found bushlike glial proliferation in the molecular layers of the cerebellum, more obvious than the usual nodule formation. Dawydowskie<sup>45</sup> agreed with Spielmeyer that the nodules were composed chiefly of neuroglia and could be found independent of vessels. He reported observations in 70 cases. The nodules were most numerous in the medulla and were present as late as thirty days after the onset of the illness. Hassin<sup>56</sup> observed that most of the lesions were associated with the smaller vessels. He described the vascular changes as consisting of congestion, thrombosis, proliferation of endothelial and adventitial cells and perivascular infiltration, especially of plasma cells. The meninges were diffusely infiltrated by various hematogenous cells.

47. Fraenkel, E.: *München. med. Wchnschr.* **62**:805, 1915.

48. Aschoff, L.: *Med. Klin.* **11**:798, 1915.

49. Jarisch, A.: *Deutsches Arch. f. klin. Med.* **126**:270, 1918.

50. Albrecht, H.: *Centralbl. f. allg. Path. u. path. Anat.* **27**:247, 1916.

51. Herzog, G.: *Centralbl. f. allg. Path. u. path. Anat.* **29**:97, 1918.

52. Abrikossoff: *Virchows Arch. f. path. Anat.* **240**:281, 1922.

53. Krinitzki, cited by Dawydowskie.<sup>45</sup>

54. Spielmeyer, W.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **47**:1, 1919.

55. Lisen, E.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **53**:199, 1919-1920.

56. Hassin, G. B.: *Arch. Neurol. & Psychiat.* **11**:121, 1924.

Although the characteristic nodules have been observed in all parts of the central nervous system, they occur more frequently in some areas than in others. According to a majority of authors, they are most often seen in the gray matter — the cortex and the various nuclei. They are especially numerous in the brain stem in the region of the olfactory and cranial nerve nuclei. Nodules have been reported in the cord by Spielmeyer,<sup>54</sup> Marinesco<sup>57</sup> and Herzog,<sup>51</sup> while Dawydowskie,<sup>45</sup> Morgenstern,<sup>58</sup> Marinesco,<sup>57</sup> Popoff<sup>57</sup> and Gutmann<sup>59</sup> have reported vascular and proliferative changes within the peripheral nerves.

The typical lesions of typhus first make their appearance around the third and fourth days of the illness. They rapidly increase, and the maximum reaction seems to occur during the second and third weeks. According to Krinitzki,<sup>58</sup> the height of the nodule formation is from the tenth to the sixteenth day, following which they gradually disappear. Nicol<sup>60</sup> found lesions to persist as long as the eighth week, while Jarisch<sup>40</sup> and Dawydowskie<sup>45</sup> saw no lesions after the eighth week in their cases.

Endemic, or murine, typhus differs from epidemic typhus chiefly on epidemiologic grounds. It is transmitted from rat to rat and from rat to man by rat fleas. Clinically it is much the same as the epidemic form except that the symptoms are less severe and the complications are few. The pathologic studies of the central nervous system in endemic typhus have been limited to examinations of experimental animals. Mooser<sup>61</sup> found typical typhus nodules in the brains of guinea pigs infected with rickettsias of Mexican typhus. Similar findings were reported by Dyer and his associates.<sup>62</sup>

*Rocky Mountain Spotted Fever.*—The diseases caused by Rickettsia rickettsii, which is transmitted from the animal reservoir to man by various species of ticks, have a wide distribution and include Rocky Mountain spotted fever of North America, Tobia fever of Colombia, São Paulo exanthematic typhus of Brazil, fievre boutonneuse of the Mediterranean countries, Kenya typhus in Africa and probably South African tick bite fever and tick typhus of India. The clinical features of Rocky Mountain spotted fever are similar to those of typhus, the differences being in the nature of the rash and the duration of the fever. Disturbances of the central nervous system are generally regarded as being severe, although there have not been reported the variety of symp-

57. Marinesco, G.: Ann. Inst. Pasteur **36**:209, 1922.

58. Morgenstern: Virchows Arch. f. path. Anat. **238**:227, 1922.

59. Gutmann, A.: Deutsche med. Wochenschr. **42**:1538, 1916.

60. Nicol, K.: Beitr. z. path. Anat. u. z. allg. Path. **65**:120, 1919.

61. Mooser, H.: J. Infect. Dis. **43**:241, 1928.

62. Dyer, R. E.; Ceder, E. T.; Lillie, R. D.; Rumreich, A., and Badger, L. F.: Pub. Health Rep. **46**:2481, 1931.

toms found with typhus (Rumreich, Dyer and Badger<sup>63</sup>; Toomey<sup>64</sup>; Baker<sup>65</sup>; Smith and Reinhard<sup>66</sup>; Bennett<sup>67</sup>; Palatucci and Maragoni<sup>68</sup>).

The pathologic aspects of Rocky Mountain spotted fever have been described in detail by Lillie.<sup>69</sup> The fundamental lesion is vascular and is characterized by endothelial swelling and proliferation, sometimes going on to necrosis and thrombosis. There is often independent perivascular cellular infiltration. Rickettsias can frequently be seen in the swollen endothelial cells. The organs most involved are the skin, the heart, the kidney and the brain. In the brain pathologic changes are not prominent until after the tenth day of the disease. This accounts for the negative findings in the rapidly fatal cases reported by Wilson and Chowning,<sup>70</sup> Le Count<sup>71</sup> and Wolbach.<sup>72</sup>

Gross examination of the brain generally reveals more or less marked vascular congestion and infection of the meningeal vessels. In 4 of 18 cases reported by Lillie<sup>73</sup> there were numerous punctate hemorrhages in the parenchyma.

The microscopic lesions described by Lillie<sup>73</sup> were of a focal character and of three general types. There was, first, lymphocytic cellular exudation in the sheaths of the smaller vessels, associated with proliferative endarteritis and occasional perivascular bleeding. Second there were perivascular and parenchymal glial nodules. Many of the nodules contained a necrotic center forming a small granuloma. Third, there was actual vascular thrombosis, associated with demyelination or necrosis of the perivascular parenchyma. Lesions of the third type were most numerous in the white matter and in the region of the brain stem. Materials from the cases reported by Pinkerton and Maxcy<sup>74</sup> and by Harris<sup>75</sup> were examined by Lillie, and the lesions were the same as noted in his series. Florman and Hofkenschiel<sup>76</sup> observed, besides the microinfarcts and glial nodules, many small perivascular accumulations

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- 63. Rumreich, A.; Dyer, R. E., and Badger, L. F.: Pub. Health Rep. **46**:470, 1931.
  - 64. Toomey, N.: Ann. Int. Med. **5**:1296, 1932.
  - 65. Baker, G. E.: Ann. Int. Med. **17**:247, 1942.
  - 66. Smith, E. B., and Reinhard, E. H.: J. Missouri M. A. **40**:166, 1943.
  - 67. Bennett, T. B.: M. Bull. Vet. Admin. **17**:425, 1941.
  - 68. Palatucci, O. A., and Maragoni, B. A.: Bull. U. S. Army M. Dept., 1944, no. 79, p. 116.
  - 69. Lillie, R. D.: Pub. Health Rep. **46**:2840, 1931.
  - 70. Wilson, L. B., and Chowning, W. M.: J. Infect. Dis. **1**:31, 1904.
  - 71. LeCount, E. R.: J. Infect. Dis. **8**:421, 1911.
  - 72. Wolbach, S. B.: J. M. Research **41**:1, 1919.
  - 73. Lillie, R. D.: Pub. Health Rep. **46**:2840, 1931.
  - 74. Pinkerton, H., and Maxcy, K. F.: Am. J. Path. **7**:95, 1931.
  - 75. Harris, P. N.: Am. J. Path. **9**:91, 1933.
  - 76. Florman, A. L., and Hofkenschiel, J.: Bull. Johns Hopkins Hosp. **66**:601, 1936.

of monocytes. Hassin<sup>77</sup> reported an aseptic type of meningitis and subarachnoid collections of lipoid-containing histiocytes. The ganglion cells throughout the brain exhibited slight swelling and chromatolysis. Lipoid granules were observed in the cytoplasm of the ganglion cells, in oligodendrocytes and ependymal cells and in the adventitial spaces about the small blood vessels. A similar fatty degeneration of the ganglion cells was reported by Scheinker.<sup>78</sup>

The pathologic changes observed in experimental Rocky Mountain spotted fever are similar to those found in the human disease (Lillie<sup>69</sup>).

São Paulo exanthematous typhus of Brazil is both clinically and pathologically identical with North American Rocky Mountain spotted fever (Dias and Martins<sup>79</sup>; de Toledo Piza, Meyer and Salles Gomes<sup>80</sup>). Topping, Heilig and Naidu<sup>81</sup> have stated that tick typhus in India resembles the North American disease. Fièvre boutonneuse is a somewhat milder disease, and no pathologic studies of human cases are available.

*Tsutsugamushi Fever.*—Tsutsugamushi fever (scrub typhus) is caused by Rickettsia tsutsugamushi (Rickettsia nipponica, Rickettsia orientalis) and is transmitted to man by several species of larval mites of the genus *Trombicula*. It has been recorded from Japan, Formosa and Korea, the Malay States, the Philippines, Australia, India, Indo-China, Sumatra and from many of the islands in the South Pacific.

Clinically it resembles other rickettsial diseases. Symptoms ascribable to involvement of the nervous system form an important part of the clinical picture (Ahlm and Lipschutz<sup>82</sup>; Lewthwaite and Savoor<sup>83</sup>; Lipman and co-workers<sup>84</sup>; Reynes and Richard<sup>85</sup>; Heaslip<sup>86</sup>). Hay<sup>87</sup> in a study of 50 patients found early and intense headache in 45, while all the patients suffered from drowsiness and extreme prostration.

The changes in the nervous system were first adequately examined by Lewthwaite,<sup>88</sup> in 1936. Gross examination in 12 cases showed sub-

77. Hassin, G. B.: Arch. Neurol. & Psychiat. **44**:1290, 1940.

78. Scheinker, I. M.: Arch. Path. **35**:583, 1943.

79. Dias, E., and Martins, A. V.: Am. J. Trop. Med. **19**:103, 1939.

80. de Toledo Piza, J.; Meyer, J. R., and Salles Gomes, L.: Typho exanthemático de São Paulo, São Paulo, Sociedade Impressora Paulista, 1932.

81. Topping, N. H.; Heilig, R., and Naidu, V. R.: Pub. Health Rep. **58**:1208, 1943.

82. Ahlm, C. E., and Lipschutz, J.: J. A. M. A. **124**:1095, 1944.

83. Lewthwaite, R., and Savoor, S. R.: Lancet **1**:255, 1940.

84. Lipman, B. L.; Byron, R. A., and Casey, A. V.: Bull. U. S. Army M. Dept., 1944, no. 72, 63.

85. Reynes, V., and Richard, J.: Bull. Soc. path. exot. **33**:70, 1940.

86. Heaslip, W. G.: M. J. Australia **1**:380, 1941.

87. Hay, C. P.: J. Roy. Nav. M. Serv. **30**:127, 1944.

88. Lewthwaite, R.: J. Path. & Bact. **42**:23, 1936.

dural hemorrhage in 2 and congestion of the brain surface in 6. In 7 cases tissues were examined microscopically. The findings were meager, but the lesions resembled those seen in Rocky Mountain spotted fever and in typhus. In some cases there were few lesions visible, and in 2 cases no diagnostic changes were observed. The most frequent lesion was perivascular proliferation of neuroglia, which in some areas actually formed tiny nodules. The larger nodules also contained lymphoid elements, pyknotic fragments of nuclei and degenerate erythrocytes. Many vessels showed proliferative endarteritis with heavy pigmentation of the endothelial cells. Clumps of rickettsias were observed in the cytoplasm of the endothelial cells in 5 of the 7 cases. Small perivascular hemorrhages were frequent. The changes were most numerous in the pons and the medulla and least numerous in the cerebellum. Kouwenaar<sup>89</sup> in 1940 found many small foci of round cell infiltration around the smaller vessels, with the infiltrates penetrating into the walls of the vessels, resulting in necrosis of the intima and subsequent thrombosis. Corbett<sup>90</sup> in 1943 described the brain changes in 4 cases. He considered the perivascular proliferation of glial cells and the lymphocytic infiltration as the most characteristic lesion in this disease. The lesions were most readily observed in the pons but were found also in the cerebrum, the cerebellum and the medulla.

Kouwenaar and Wolff<sup>91</sup> in a description of mite fever in the guinea pig noted lesions of the brain similar to those found in one half of their cases of typhus.

*Q Fever.*—Q fever (Queensland fever, quadrilateral fever) was first described from Australia in 1937 by Derrick<sup>92</sup> and from the United States in 1938 by Dyer.<sup>93</sup> It is caused by Rickettsia burneti, which is transmitted from the natural animal reservoir to man by infected ticks. Although in this disease there are symptoms which suggest involvement of the central nervous system, there is at present no pathologic evidence in man to substantiate such an impression. Most of the studies of human Q fever are limited to the pulmonary changes. Lillie<sup>94</sup> studied the nervous system changes in guinea pigs infected with rickettsias of Q fever. He observed perivascular lymphocytic infiltration and proliferation of the vascular endothelium, as well as a few glial nodules. He stated that "compared with endemic and European typhus, or even with Rocky Mountain spotted fever, focal brain and cord lesions in the guinea pig are strikingly infrequent."

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89. Kouwenaar, W.: Geneesk. tijdschr. v. Nederl.-Indië **80**:1119, 1940.
  90. Corbett, A. J.: Bull. U. S. Army M. Dept., 1943, no. 70, p. 34.
  91. Kouwenaar, W., and Wolff, J. W.: J. Infect. Dis. **55**:315, 1934.
  92. Derrick, E. H.: M. J. Australia **2**:281, 1937.
  93. Dyer, R. E.: J. A. M. A. **122**:331, 1943.
  94. Lillie, R. D.: Pub. Health Rep. **57**:296, 1942.

*Trench Fever.*—Trench fever (Wolhynian fever, five day fever) is a specific louse-borne disease which seems to be associated with war. The exact nature of the etiologic agent has not been determined, although there is considerable evidence that it may prove to be a rickettsia. Although many reports indicate that the central nervous system is involved, most patients recover and no pathologic studies of the affected nervous system are available at present.

#### BACTERIAL DISEASES

*Bartonellosis.*—Bartonellosis, Oroya fever or verruga peruana is a disease peculiar to Peru, although recently cases have been reported in Colombia and in Ecuador (Camargo<sup>95</sup>). It has been recognized for many years and has appeared in two clinical forms, namely, as a severe, highly fatal febrile anemia (Oroya fever) and as a cutaneous eruption (verruga peruana). For many years it was doubtful whether the aforementioned two conditions were related. In 1885 Daniel A. Carrión vaccinated himself with blood from a Peruvian wart, and in twenty-one days Oroya fever developed, from which he died, thus proving the interrelationship of the two conditions. The causative organism was isolated in 1902 by Barton.<sup>96</sup> In 1913 it was assigned to the genus *Bartonella* and is considered to be midway between a rickettsia and a bacillus.

Carrión<sup>97</sup> first called attention to the frequent involvement of the nervous system. The neurologic manifestations are most variable, since the organism is capable of affecting all parts of the nervous system. Characteristically, various clinical forms of neurobartonellosis have been described, namely: (1) hypertensive, (2) meningeal, (3) convulsive, (4) apoplectiform, (5) extrapyramidal and (6) mental.

The histologic nature of the cerebral lesions has been described in detail by Mackehenie and Alzamora<sup>98</sup> and by Lastres.<sup>99</sup> The chief involvement is vascular, implicating primarily the capillaries, the pre-capillaries and the small venules. Apparently the first lesions involve the endothelial cells, which are invaded by the organisms and undergo swelling and proliferation, producing partial or even complete vascular occlusion. Many of these involved endothelial cells break off into the vessel, filling the lumen with parasitized cells. This produces an occlusive thrombus, with the result that focal areas of tissue degeneration develop, with destruction of many of the neighboring cell elements. The nerve cells adjacent to these occluded vessels reveal many acute

95. Camargo, L. P.: Fac. de med., Bogotá **9**:160, 1940.

96. Barton, A. L.: Crón. méd., Lima **18**:193, 1902.

97. Carrión, D. A., cited by Lastres.<sup>99</sup>

98. Mackehenie, D., and Alzamora, V. V.: J. neuro-psiquiat. panam. **2**:166, 1940.

99. Lastres, J. B.: Rev. méd. peruana **6**:1690, 1934.

changes, consisting of swelling, chromatolysis and even fragmentation and disappearance of nerve cells. Within the white matter there occur foci of necrosis, invaded by fat granule cells. As a result of this ischemia, the vessels themselves undergo weakening, with red cells and some leukocytes escaping into the perivascular spaces and even beyond into the injured brain tissue. Another very prominent alteration consists in the appearance of tiny granulomas adjacent to many of the involved vessels. These granulomas result from focal adventitial proliferation associated with rapid multiplication of local histiocytes and monocytes to produce a nodule composed chiefly of mesenchymal elements. They also contain scattered lymphocytes, plasma cells and many multinucleated elements. They are observed chiefly in the gray substance of the brain (Lastres<sup>100</sup>) and vary in frequency from case to case. A few scattered vessels contain no real granulomas but tiny collections of round cells within their adventitial layers or within the perivascular spaces. Around many of the ischemic foci within the white matter appear tiny foci of glial proliferation producing glial rosettes. At no time is this glial response of a diffuse nature.

In the more chronic process the granulomas undergo fibrosis, which leaves fibrous tissue scars adjacent to the vessels.

The lesions in neurobartonellosis are extremely widespread and have been observed within all regions of the nervous system. Mackehenie and Alzamora<sup>98</sup> in a thorough pathologic study observed extensive changes not only within the brain but also within the sympathetic nervous system, the peripheral nerves and the pituitary gland. The brachial plexus revealed the typical vascular occlusions and granulomas involving the perineural vessels. There was monocytic exudation among the nerve fasciculi. The pituitary gland showed blood vessels occluded by parasitized elements, with zones of necrosis and disorganization of the glandular acini resulting. Granulomas were present within both the anterior and the posterior lobe of this gland. Bartonellas have been observed within the dilated vessels of the choroid plexus as well as within the astrocytes and the microglia (Lastres<sup>100</sup>; Monge and Mackehenie<sup>101</sup>).

Monge and Mackehenie in a series of cases observed old clotted blood covering the hemispheres in 1 case and numerous punctate hemorrhages in 1. In all cases there was revealed marked vascular congestion on gross inspection. Histologically the typical endothelial proliferation, occlusive thrombosis and granulomatous formations were revealed in all the brains.

*Bacillary Dysentery.*—Bacillary dysentery is an acute infectious disease caused by *Bacillus dysenteriae* and resulting primarily in gastro-

100. Lastres, J. B.: *América clin.* 5:11, 1943.

101. Monge, C., and Mackehenie, D.: *Rev. médica peruana* 4:523, 1932.

intestinal symptoms. The disease is acute in onset and terminates either abruptly in death after four to six days or more slowly in gradual recovery. The disease is more virulent in the tropics, where it appears in epidemic form (India, Indo-China, Japan, Java, Northern Brazil, Egypt, Palestine and other areas). In the United States and northern Europe the outbreaks are small and often sporadic, being most frequent in hospitals for patients with mental diseases and in orphanages and among troops during war. The outbreaks seem influenced primarily by sanitation rather than by geographic distribution since the disease is spread chiefly by contaminated food and drink or by carriers convalescing from the disease. The organism was first isolated in 1898, by Shiga,<sup>102</sup> in Japan. Since then it has been recognized that *B. dysenteriae* comprises a large group of micro-organisms (Flexner<sup>103</sup>; Strong and Musgrave<sup>104</sup>; Kruse<sup>105</sup>; Hiss and Russell<sup>106</sup>). At present all dysentery bacilli are divided into two large groups, namely, those that produce no acid on mannite sugar and no indole (Shiga-Kruse type) and those that do produce acid on mannite and do form indole (Flexner-Hiss-Strong type).

Dysentery bacilli are usually present in the gastrointestinal tract, where ulceration results. Even in the more severe lesions the organisms remain localized to the bowel, rarely invading the blood stream. Since the bacilli remain so well localized, it is generally believed that the cerebral complications are secondary in type and probably result from toxic circulatory or metabolic factors. The frequency of complications referable to the nervous system varies with the severity of the infection. Zellweger<sup>107</sup> in a study of cases observed such complications in 32.7 per cent, while Gröer<sup>108</sup> reported involvement of the nervous system in 56 per cent of his 50 cases. Symptoms referable to the nervous system occur chiefly in children and are not infrequent in patients with profound acute and chronic dysentery (Zellweger<sup>107</sup>; Alexander and Wu<sup>109</sup>). The clinical picture may be variable. It often resembles that of epidemic encephalitis except that it is preceded or accompanied by a specific type of dysentery. The peripheral nervous system may also be involved. This complication occurs chiefly in adults during the period from the second to the fourth week of illness and may take the form

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- 102. Shiga, K.: *Zentralbl. f. Bakt. (Abt. 1)* **24**:817, 870 and 913, 1898.
  - 103. Flexner, S.: *Bull. Johns Hopkins Hosp.* **11**:231, 1900.
  - 104. Strong, R. P., and Musgrave, W. E.: *J. A. M. A.* **35**:498, 1900.
  - 105. Kruse, W.: *Deutsche med. Wochenschr.* **26**:637, 1900.
  - 106. Hiss, P. H., and Russell, F. F.: *J. M. Research* **13**:1, 1904.
  - 107. Zellweger, H.: *Praxis* **32**:707, 1943.
  - 108. von Gröer, F.: *Ztschr. f. Kinderh.* **21**:220, 1919.
  - 109. Alexander, L., and Wu, T. T.: *Chinese M. J.* **48**:1, 1934.

of mononeuritis or peripheral neuritis (Müller-Deham<sup>110</sup>; Singer<sup>111</sup>; Alexander and Wu<sup>109</sup>; Zellweger<sup>107</sup>).

Only scattered reports describing the changes in the nervous system in man or even in experimental animals are available. Probably the most extensive studies have been reported by Alexander and Wu.<sup>112</sup> They studied 16 cases of bacillary dysentery and reported what they called "circulatory and regressive parenchymal changes" but no inflammatory alterations. Sporadic ischemic foci were present throughout the cortical gray matter, involving chiefly the third to fifth cortical layers and located near small vessels. These areas showed deficiency of nerve cells or alteration of numerous neurons of varying degree and type. Some cells were pyknotic, while others had lost their staining properties. Many of these foci were replaced by proliferated glial elements. Many of the neurons unassociated with these ischemic foci were pyknotic and revealed chronic alteration of the nerve cells, even being replaced or surrounded by glial rosettes. With Bielschowsky stains, these cells showed argentophilia with thickening, breaking and crumbling of the intracellular neurofibrillae and argentophilia of the nuclei and the glial cells. The cerebellar cortex revealed damage of the Purkinje cells with formation of glial shrubs along the dendrites and about the damaged cell. In 1 case there was complete ischemic necrosis of the cerebellar cortex with focal destruction of the granular layer and the Purkinje cells. Glial nodules were scattered throughout, unassociated with the ischemic areas. In only 1 case was lymphocytic infiltration observed within the white matter, chiefly of perivascular distribution.

The remaining reports within the literature describe changes much less extensive. Buttenwieser<sup>113</sup> reported a case of Shiga-Kruse dysentery in which death occurred two weeks after the onset of illness. At autopsy numerous ring and ball hemorrhages were observed throughout the cortex, the basal ganglia and the midbrain. Oesterlin<sup>114</sup> in autopsy studies on 11 cases of dysentery observed significant changes in only 1. The patient was a 17 month old child who died following a period of motor unrest, vomiting and bloody diarrhea. At autopsy changes were observed chiefly in the cerebellum. The molecular layer contained an occasional glial nodule. In certain areas there was shrub-like proliferation of glia about the Purkinje cells. A diffuse glial increase was also observed, involving chiefly the granular and inner molecular layers. Spielmeyer<sup>115</sup> was the only investigator to describe

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110. Müller-Deham, A.: Wien. med. Wchnschr. **65**:654, 1915.
  111. Singer, K.: Monatschr. f. Psychiat. u. Neurol. **41**:245, 1917.
  112. Alexander, L., and Wu, T. T.: Arch. Neurol. & Psychiat. **33**:72, 1935.
  113. Buttenwieser, S.: München. med. Wchnschr. **76**:1472, 1920.
  114. Oesterlin, E.: Ztschr. f. d. ges. Neurol. u. Psychiat. **88**:323, 1924.
  115. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **123**:161, 1930.

small necrotic foci around small blood vessels of the white matter in dysentery.

A few reports are available illustrating experimental lesions, chiefly those in rabbits. Lotmar,<sup>116</sup> using the Shiga-Kruse toxin, observed changes involving all parts of the nervous system. The lesions were of two types. Some were focal in nature and consisted of areas of devastation in which all cell elements were degenerated. These foci often were surrounded by a mild glial increase. In certain foci the neurons were only partially destroyed and showed marked granular degeneration. The second type of lesions consisted of "irritative encephalitides." These were comprised of focal collections of cells, chiefly glia cells intermixed with fat granule cells and plasma cells. Vessels in the involved areas showed intensive lymphocytic infiltrations and some endarteritis. These two types of lesions occurred independently of each other in different animals. Guggisberg,<sup>117</sup> using 20 rabbits and intravenous injections, found no focal lesions and only diffuse neuronal involvement, consisting of tigrolysis, nuclear disintegration, glial increase, but no vessel changes. Tupa<sup>118</sup> and Karasawa<sup>119</sup> observed alterations, limited chiefly to the spinal cord. The lesions seemed to localize within the cervical cord and bulb and appeared less commonly within the lumbar regions. Isolated lesions were observed by Tupa within the thalamus, the olfactory lobes and the cerebellum. In the cord the gray matter was chiefly involved. Hyperemia and hemorrhages were common. The anterior horn cells were swollen, chromatolytic and even vacuolated. There was only mild glial increase.

*Cholera.*—Cholera is an infectious disease caused by *Vibrio comma* and involves primarily the intestinal tract. These vibrios are spread chiefly by contaminated food and water. The disease has been endemic in many parts of the world (India, Asia and the Far East). Six worldwide pandemics have been traced to India, and all except the first spread throughout Europe, producing tremendous devastation. Most of the studies in the complications referable to the central nervous system are based on studies of cases resulting during the outbreak of 1891, which produced over a million deaths in Russia alone.

Encephalic complications are almost never mentioned in the usual descriptions of cholera, in spite of their frequency during the larger pandemics. Often the clinical symptoms referable to the nervous system are so mild as to be entirely overlooked. The patient's face becomes

116. Lotmar, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. 8:345, 1911.

117. Guggisberg, H.: Arb. a. d. Inst. z. Erforsch. d. Infektions-krankh. in Bern, 1908, p. 51.

118. Tupa, A.: Compt. rend. Soc. de biol. 92:1141, 1925.

119. Karasawa, M.: Ztschr. f. Immunitätsforsch. u. exper. Therap. 6:390, 1910.

drawn and haggard; sleep becomes disturbed and thinking difficult. In the more severe type there may occur vertigo, headaches, restlessness, convulsions, apathy progressing to stupor or severe delirium (Gavino<sup>120</sup>; Weber and Ranke<sup>121</sup>; Delasiauve<sup>122</sup>; Burq<sup>123</sup>; Obregia and Pitulesco<sup>124</sup>; Kraepelin<sup>125</sup>; Mesnet<sup>126</sup>; Ball<sup>127</sup>).

Only scattered studies are available on the changes in the central nervous system affected by cholera, and many of these are in the earlier Russian literature, which was not available to us. Most of the studies appeared following the European pandemic of 1891, particularly in the German and the Russian literature. In 1873 Iwanowsky<sup>128</sup> reported diffuse cortical changes. The cortical neurons showed swelling and some tinctorial loss. Scattered cells became smaller, and each was surrounded by an enlarged pericellular space. The blood vessels of the cortex were congested, and their endothelium was swollen and often filled with fat. Changes of the types noted, involving chiefly the cortical neurons and the vessels, appear to be fairly constant and have been recorded repeatedly. Tizzoni and Cattani<sup>129</sup> in 1888 studied the brains of 54 persons dying in Bologna, Italy, in 1886 and found changes similar to those of Iwanowsky. They also described some cellular chromatolysis and dilatation of the perivascular spaces. They were able to isolate V. comma from the spinal fluid of 2 of their patients. In 1894 Popoff<sup>130</sup> reported his observations on 2 patients dying in Warsaw, Poland. Again the most striking changes occurred within the cortical neurons, which were diffusely and often severely injured. Most of the cells showed swelling, chromatolysis and eccentricity of the nuclei. In many, however, even fragmentation had occurred. The nuclei showed no changes in spite of the severity of the cell damage. The blood vessels were congested and showed marked endothelial swelling and proliferation. Some of the larger vessels contained small yellowish masses both within their walls and within their perivascular spaces. Popoff reported a diffuse glial increase throughout both the gray and the white matter, particularly in the patients whose disease had become chronic. Tschistowitch<sup>131</sup>

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- 120. Gavino, C.: J. Philippine Islands M. A. **7**:3, 1927.
  - 121. Weber and Ranke: Lancet **2**:344, 1853.
  - 122. Delasiauve, M.: Ann. méd.-psychol. **13**:331, 1849.
  - 123. Burq, M. V.: Gaz. méd. de France **5**:82, 1850.
  - 124. Obregia and Pitulesco: Encéphale **9**:393, 1914.
  - 125. Kraepelin, E.: Arch. f. Psychiat. **12**:322, 1882.
  - 126. Mesnet, E.: Ann. méd.-psychol. **30**:317, 1866.
  - 127. Ball, M. B.: Encéphale **1**:30, 1885.
  - 128. Iwanowsky, N., cited by Tschistowitch.<sup>131</sup>
  - 129. Tizzoni, G., and Cattani, G.: Beitr. z. path. Anat. u. z. allg. Path. **3**:191, 1888.
  - 130. Popoff, N. M.: Virchows Arch. f. path. Anat. **136**:42, 1894.
  - 131. Tschistowitch, T. J.: Virchows Arch. f. path. Anat. (supp.) **144**:40, 1896.

in 1896 reported on 21 cases, and his findings were similar to those of Popoff although he noted no glial increase. The nerve cells in Tschistowitch's cases were often severely implicated even to the stage of vacuolation. In very severe long-standing cholera the cell damage was often so severe that large areas of cortex were filled with vacuolated, fragmented, granular cytoplasmic masses. The motor cortex and the cerebellum were uninvolved. The vascular congestion and endothelial proliferation were also often marked, with some of the vessels being surrounded by perivascular bleeding. In an occasional subacute case of the form of the disease the author observed markedly widened perivascular spaces, often containing leukocytes.

One of the most recent studies of cases of cholera was published in 1922 by Pines.<sup>132</sup> In these cases, also, the chief alterations were limited to the cortex with diffuse destruction of the nerve cells. There appeared to be progressive enlargement of the pericellular and perivascular spaces. These spaces continued to enlarge so that in the more chronic processes, the entire cortex was filled with numerous tiny cavities, some of which were filled with amorphous masses or degenerated nerve cells. The author observed no changes within the white matter or the cerebellum.

The spinal cord also becomes involved in Asiatic cholera. Popoff<sup>130</sup> observed some changes within the anterior horn cells, which were at times vacuolated and filled with yellowish granules. Scattered cells contained more than one nucleus. In the white matter there occurred swelling of the axis-cylinders, particularly in the lateral columns. Michailow<sup>133</sup> studied the cord in 2 fatal cases of cholera and observed scattered clumps of *V. comma* in selected areas of the cord. The anterior horn cells in the regions where the micro-organisms were found showed complete chromatolysis, while the neurons at other levels were uninvolved. In 1912 Michailow<sup>134</sup> again reported on the cord changes in 8 cases of cholera. In the acute illness no changes were visible. The most constant alteration consisted of myelin degeneration around the periphery of the cord. In 2 cases some scattered demyelination had occurred also within the lateral and the posterior columns. The author felt that the latter changes were secondary to axonal degeneration following damage of cortical nerve cells.

The etiologic explanation of these changes in the central nervous system has been the subject of considerable discussion. Both Michailow<sup>134</sup> and Tizzoni and Cattani<sup>129</sup> isolated *V. comma* from the spinal fluid and from cord tissue and held that the changes were produced by the organisms themselves and that these reached the nervous system

132. Pines, I. L.: Arch. f. Psychiat. **66**:796, 1922.

133. Michailow, S.: Zentralbl. f. Bakt. (Abt. 1) **62**:545, 1912.

134. Michailow, S.: Zentralbl. f. Bakt. (Abt. 1) **50**:296, 1909.

by way of the blood stream. This theory receives some support from the fact that *V. comma* has been demonstrated in the blood by many investigators. On the other hand, bacteremia is rare in cholera, and the organism does have a tendency to remain localized to the intestine. Most investigators (e. g. Tschistowitch<sup>131</sup> and Stitts and Strong<sup>135</sup>) have felt that *V. comma* produces a powerful endotoxin, which is set free when the vibrio undergoes disintegration. It is the absorption of this toxin that produces the severe complications referable to the nervous system. Finally, Kraepelin<sup>125</sup> believed that the toxin of cholera rarely reaches the brain. He thought that most of the encephalic involvement was secondary to anoxia, which was produced by the severe diarrhea and dehydration with subsequent high concentration of blood.

*Bubonic Plague*.—Bubonic plague is a specific infectious disease affecting man and some of the lower animals. It is produced by a short round rod, *Bacillus* or *Pasteurella pestis*. The classic form of this disease, as the name indicates, manifests itself after an initial period of generalized symptoms by painful swellings with effusions into the lymphatic glandular tissues chiefly of groin, armpit and neck, resulting in the appearance of the classic buboes. This disease may manifest itself in a pneumonic and a septicemic form, in which the buboes do not occur or, if they do, appear only as a late manifestation of the disease. Other forms have been described, such as carbuncular, intestinal and cerebral forms (Simpson,<sup>136</sup> Choksy<sup>137</sup>). Plague may manifest itself in sporadic, epidemic and pandemic modes and probably has caused more deaths in the world than any other human illness.

This disease is one of great antiquity and was well recognized in Syria and its vicinity three thousand years ago. The disease prevailed in endemic and epidemic modes within the countries on the southern and eastern shores of the Mediterranean (Libya, Egypt, Syria), spreading periodically over most of Europe (sixth, eleventh and fourteenth centuries.) In the United States the first infection reached San Francisco from Honolulu, Hawaiian Islands, in 1900. By 1907 it had spread over the entire state (Lloyd<sup>138</sup>; Kellogg<sup>139</sup>). In 1924, 32 cases of plague were identified in Los Angeles, and by 1942 the disease had appeared in the states of Nevada, Utah, North Dakota and Wyoming. According to Lloyd, the endemic center in the United States was the California ground squirrel. He surmised that plague would remain a menace to inhabitants throughout the world indefinitely.

135. Strong, R. P.: *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*, Philadelphia, The Blakiston Company, 1943, vol. 1, p. 602.

136. Simpson, W. J.: *A Treatise on Plague*, Cambridge, University Press, 1905.

137. Choksy, K. B.: *Am. J. M. Sc.* **138**:351, 1909.

138. Lloyd, B. J.: *J. A. M. A.* **85**:729, 1925.

139. Kellogg, W. H.: *Am. J. Pub. Health* **10**:599, 1920.

The causative organism of plague was first described by Yersin, in 1894. It is a short round or oval rod with bipolar staining. In the living body the bacilli are abundant in buboes, as shown by smears, in the sputum if the infection is pneumonic and in the blood if septicemia develops. At autopsy the organisms can be obtained from many organs, including the brain, and from the spinal fluid (França<sup>140</sup>).

Next to the buboes and the pneumonic lesions, the encephalic changes comprise the most common disturbance. Since the causative organism is found within the brain, the involvement of this organ is a primary one and constitutes a primary encephalitis. Symptoms of such involvement are therefore extremely common in all forms of plague and frequently constitute the predominating symptomatic aspect. Usually these symptoms are of a diffuse rather than a focal nature, indicating a widespread involvement of the nervous system. Cerebral complications occur most frequently in the bubonic form of the disease, in which the course is a little more prolonged.

The chief neurologic complaints are headaches and vertigo, which increase in severity. As the disease progresses, the severe toxemia affects the intellect more and more, producing mental dulness, confusion and delirium. Terminally the patient shows increasing lethargy and coma. In the septicemic form, because of the overwhelming infection of the blood stream, the cerebral symptoms develop with great rapidity, and the course of the illness may be short.

Most of the general symptoms no doubt are due to the extreme toxemia occurring in plague. Since the bacillus of plague actually localizes within the meninges or the brain substance, one would expect focal symptoms to accompany the more general involvement already described. The most characteristic of these focal phenomena are ataxic gait and incoordination of speech (Wu Lien-Teh<sup>141</sup>; Simpson<sup>136</sup>).

It is surprising that there have been so few detailed pathologic studies of plague reported, particularly since it is generally agreed that the nervous system is one of the organs chiefly involved. Since this illness is largely a disease of the lymphatic and the vascular system through which the bacilli of the disease and their toxins are brought in contact with nearly every part of the body, it would be expected that the chief lesions within the brain would be centered about the vessels. Edema, congestion and hemorrhage within the meninges and the brain tissue have been frequently reported (Wu Lien-Teh<sup>141</sup>; Stitts and Strong<sup>142</sup>). Stitts and Strong described petechiae as occurring primar-

140. França, C.: Névraxe 1:321, 1900.

141. Wu Lien-Teh.: A Treatise of Pneumonic Plague, Publication of the League of Nations, Geneva, Switzerland, 1926, vol. 3.

142. Strong,<sup>135</sup> p. 688.

ily within the meninges, the mesencephalon and the medulla. Calmette and Salimbeni<sup>143</sup> and Crowell<sup>144</sup> described meningitis primarily in their fatal cases. In both of Crowell's cases at autopsy the cerebrospinal fluid was turbid and the subarachnoid space and the ventricles were covered with a thick, stringy yellow pus which was loaded with plague bacilli. Calmette and Salimbeni described 2 fatal cases, 1 of meningitis and 1 of meningoencephalitis. In the latter the gray matter was congested and edematous, while the subarachnoid space contained an exudate yielding plague bacilli on culture.

By far the most complete autopsy studies on plague have been reported by França<sup>140</sup> and Nepveu.<sup>145</sup> Nepveu described only mild meningitis, with many of the venules thrombosed and containing clumps of plague bacilli. The cortical vessels were frequently thrombosed and filled with bacilli. Many of the vessels were surrounded by leukocytes, this perivascular involvement decreasing within the deeper cortical layers. The cortical neurons were extensively damaged. Many were surrounded by leukocytes. These showed chromatolysis, fragmentation of the cell processes, disintegration of the cell body and destruction of the axons entering the cell. Many damaged nerve cells were unassociated with a leukocytic reaction. The damaged cells were swollen, pale, vacuolated or granular. Their nucleoli were often displaced peripherally. These neuronal changes were irregular, involved scattered cells within the cortex and were present in varying degrees of severity. The neuroglia was moderately increased. França reviewed 11 cases and found bacilli in all but 3. The bacilli were observed most frequently within the thrombosed vessels but could also be identified free within the white matter or within the cortical neurons and the adjacent tissues. The most severe neuronal alterations occurred within the cerebral cortex, the cerebellum and the medulla. The nerve cells were swollen, stained lightly and showed peripheral chromatolysis. In the more prolonged cases, the cell body was vacuolated and even fragmented, the nuclei pyknotic and nucleoli eccentrically placed. In 3 cases the most severe cellular damage appeared within the motor cortex, but in most cases the involvement was patchy, with many altered cells occurring among entirely unchanged elements. Babes<sup>146</sup> studied the effect of plague toxin on the spinal cord of the rabbit. With large doses, the animals died within two days and showed swelling, vacuolation and fragmentation of the processes of cells within the anterior horns. When smaller doses of toxin were administered, the rabbits lived for six days and revealed

143. Calmette, A., and Salimbeni, A. T.: Ann. Inst. Pasteur **12**:865, 1899.

144. Crowell, B. C.: Philippine J. Sc. **10**:249, 1915.

145. Nepveu, M. G.: Compt. rend. Soc. de biol. **49**:863, 1897.

146. Babes, V.: Klin. Wchnschr. **35**:36, 1898.

hemorrhages and leukocytic infiltration within the anterior horns. The cells of the anterior horns were only mildly altered. In the more chronic illness, the round cell infiltration increased, while the neuronal damage diminished in severity.

*Melioidosis*.—Melioidosis is a tropical disease which is localized to the extreme Orient, being found chiefly in the Malay States, Netherlands Indies, Ceylon and Indo-China. It produces in man a disease resembling glanders. This illness was first recognized by Whitmore and Krishnaswamy,<sup>147</sup> in 1912, while they were studying cadavers of vagabonds. In the following years Whitmore isolated the bacillus, which he named *Bacillus pseudomallei* and which has been renamed *Bacillus whitmori*. By 1933 the numbers of cases of melioidosis observed in the Orient totaled 95, with numerous cases occurring in Europe.

Pathologically the characteristic lesion consists of small yellowish caseous nodules which begin as focal collections of leukocytes and increase in size. The lesions appear as military granulomas with necrotic centers. They have been reported in all organs except the brain. However, the organism has been found in the blood of patients with the septicemic form of the disease, and the clinical picture of the involvement of the central nervous system certainly suggests the probability of cerebral involvement.

*Leprosy*.—Leprosy is a well known infectious disease which has been recognized in man since great antiquity. It has been endemic in Egypt, Persia, India and much of the rest of the Orient since ancient days. According to McCoy<sup>148</sup> there are 400 to 500 persons with the disease in active form in the United States. In South America there are some 30,000, particularly in Brazil.

This disease probably owes its spread to contacts occurring over a long period with those having active lesions, but the exact mode of spread or portal of entry of the infective organism is not known. There are two well recognized types of the disease. One is the nodular or cutaneous variety, characterized by granulomatous proliferation within the skin and the subcutaneous tissues forming the so-called leproma. This consists of a mass of so-called leprosy cells or foam cells which are often filled with leprosy bacilli and intermixed with various types of connective tissue. The second type is known as the maculoanesthetic or neural type and is characterized by flat thickenings within the covered regions of the body associated with areas of anesthesia within these involved regions. The nerves become thickened, and muscular palsies and atrophies develop, associated with ulcerations, contractures and severe mutilations secondary to the trophic changes.

147. Whitmore, A., and Krishnaswamy, C. S.: Indian M. Gaz. **47**:262, 1912.

148. McCoy, G. W.: Arch. Dermat. & Syph. **37**:169, 1938.

Bacillus leprae (*Mycobacterium leprae*) has been accepted as the cause of human leprosy and is present in large numbers in the characteristic lesions throughout the body as well as within the peripheral and even the central nervous system. There is some question whether the organisms occur within the circulating blood, but Stitts and Strong expressed the belief they do and probably spread in this way from organ to organ.

Occasionally during the course of the disease certain special symptoms may develop which suggest a more specific involvement of the nervous system. Many patients, especially at the beginning of their illness, show a tendency toward somnolence and complain of severe headaches. Often this somnolence is associated with frequent nightmares of a terrorizing nature, visual hallucinations and an agonizing sensation of stiffness. Motor and sensory manifestations are chiefly referable to involvement of the peripheral nervous system. Mental symptoms have been reported in many cases of leprosy (Bodros<sup>149</sup>; Jakob and Meggendorfer<sup>150</sup>; de Beurmann and co-workers<sup>151</sup>; Muir<sup>152</sup>; Jones and Pearson<sup>153</sup>; Swerbejew<sup>154</sup>). All forms of psychoses have been observed.

Naturally the appearance of an organic psychosis in a patient with leprosy, as well as the appearance of the bacilli in the circulation, make one consider the possibility of actual encephalitis with definite changes within the central nervous system. Reports of brain changes in this disease are surprisingly few. Many authors who have studied the brain in leprosy have expressed the opinion that no definite changes occur (Thoma<sup>155</sup>; Neisser<sup>156</sup>; Leloir<sup>157</sup>; Gerlach<sup>158</sup>; Rikli<sup>159</sup>). However, the bacilli of leprosy have been reported within the intracranial cavity in isolated cases. Doutrelepont and Wolters<sup>160</sup> observed them within the perivascular spaces of the pia, while Brutzer<sup>161</sup> found them in the

149. Bodros, P.: Ann. méd.-psychol. **1**:278, 1912.

150. Jakob, A., and Meggendorfer, F.: Arch. f. Dermat. u. Syph. **130**:367, 1921.

151. de Beurmann; Roubinovitch, and Gourgerot: Rev. neurol. **14**:292, 1906.

152. Muir, E.: Leprosy Rev. **10**:114, 1939.

153. Jones, R., and Pearson, R.: Lancet **179**:728, 1910.

154. Swerbejew, N.: Arch. f. Psychiat. **108**:572, 1938.

155. Thoma, R.: Virchows Arch. f. path. Anat. **57**:455, 1873.

156. Neisser, A.: Virchows Arch. f. path. Anat. **84**:514, 1881.

157. Leloir, H.: Arch. de physiol. **8**:391, 1881.

158. Gerlach, W.: Virchows Arch. f. path. Anat. **125**:126, 1891.

159. Rikli, A.: Virchows Arch. f. path. Anat. **129**:110, 1892.

160. Doutrelepont and Wolters, M.: Arch. f. Dermat. u. Syph. **34**:80, 1896.

161. Brutzer, C.: Dermat. Ztschr. **5**:750, 1898.

dura over the hypophysis. Lie<sup>162</sup> in a careful study of 5 cases observed the bacilli within the nuclei of the medulla, particularly within the facial nerve, with no clinical evidence of involvement. Lie surmised that in nodular leprosy the entire nervous system is heavily invaded but that the brain is immune against the bacilli and thus the organisms are unable to grow.

The most complete studies on the brain changes in leprosy have been published by Dwijkoff,<sup>163</sup> Vilde,<sup>164</sup> Stahlberg<sup>165</sup> and Jacob and Meggendorfer.<sup>166</sup> In most cases the changes were diffuse, involving large areas of the brain but being most marked within the basal ganglia, the midbrain and the medulla. The nerve cells showed patchy chromatolysis, with some cells having undergone complete tigrolysis, some vacuolation and definite nuclear changes with pyknosis and irregularity of nuclear outline. Dwijkoff observed some fatty degeneration of the ganglion cells. Both Vilde and Stahlberg observed cerebellar changes. The former reported definite reduction in the Purkinje cells, while the latter observed the most severe changes within the molecular layer. The vessels throughout the brain revealed marked congestion. Many of the smaller ones showed marked endothelial increase, with fatty changes involving both the endothelium and the medial elements (Dwijkoff; Vilde). Stahlberg observed some widening of the perivascular spaces but no demyelination. The reaction on the part of the glia was most variable. Stahlberg reported the glial elements as also showing degenerative changes, while Vilde observed proliferative glial changes with the production of glial rosettes. Actual presence of inflammatory elements was observed only rarely. Dwijkoff in his case observed a few vessels within the corpus striatum infiltrated with leukocytes, while Vilde reported scattered vessels within the cerebral hemispheres and the medulla encircled by perivascular lymphocytes. In 2 cases this infiltration was quite pronounced especially within the medulla and the midbrain. Vilde also observed some scattered necrotic foci with secondary glial increase. Jakob and Meggendorfer described the cerebral changes in a 25 year old man who died of leprosy. The brain contained a softened area in the right frontoparietal region, which histologically showed chronic degeneration of the parenchyma with fat granule cell invasion and glial increase. The neurons within this area were destroyed. The entire brain, however, revealed scattered changes within the nerve cells of both an acute and a chronic type. Many ganglion cells were swollen and partially chromatolytic. Some of these elements were vacuolated and surrounded by

162. Lie, H. P.: Acta path. et microbiol. Scandinav. **7**:32, 1930.

163. Dwijkoff, P. P.: Frankfurt, Ztschr. f. Path. **40**:185, 1930.

164. Vilde, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **133**:119, 1931.

165. Stahlberg, H.: Arch. f. Psychiat. **41**:596, 1906.

ameboid cells. A few nerve cells were shrunken and pyknotic. Within the white matter there was diffuse glial increase.

The spinal cord is much more frequently involved in leprosy, although the changes reported have been variable. The most constant alteration is that of the ganglion cells, chiefly those of the anterior horns. These cells were frequently filled with leprosy bacilli (Uhlenhuth and Westphal<sup>166</sup>; Shaw<sup>167</sup>). Chassiotis<sup>168</sup> expressed the opinion that the bacilli were not situated within the nerve cells but appeared, both in the gray and in the white matter, as oval bodies filled with bacilli and covered by a membrane. The earliest neuronal change within the spinal cord was a tinctorial loss, particularly around the bacilli. The affected cell became vacuolated to the point of destruction of the entire cell body. Next to the neuronal damage, one of the most consistent changes in the cord was focal degeneration of the dorsal columns (Lie<sup>162</sup>; Looft<sup>169</sup>; Saingin<sup>170</sup>; Jeanselme and Marie<sup>171</sup>) occasionally associated with involvement of Clarke's columns (Jeanselme and Marie<sup>171</sup>; Tschirjew<sup>172</sup>). In the degenerated areas, axons were only rarely seen, and the posterior rootlets often were atrophic. Etiologically this damage of the cord has been subjected to much speculation. Lie surmised that the changes were secondary to the peripheral neuritic changes and not due to primary involvement by the leprosy bacilli or its toxin. Central cavitation of the spinal cord has also been reported, but in many of the cases true syringomyelia has eventuated rather than leprosy of the cord. Pestana and Bettencourt,<sup>173</sup> however, observed a patient with a syringomyelic syndrome who at death showed lepra bacilli within the cord cavity. Leprosy frequently attacks the sympathetic ganglions and characteristic changes have been reported by many authors (Sudakewitsch<sup>174</sup>; Ermakóva<sup>175</sup>). The ganglion cells showed marked vacuolation, with many leprosy bacilli distributed between the vacuoles in the remnants of the cytoplasm. Bacilli were present within the cell capsule. The nuclei of the vacuolated cells were frequently pyknotic or entirely absent.

Changes within the peripheral nerves have been well described, and the observations require no repetition at this time.

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- 166. Uhlenhuth and Westphal, A.: *Zentralbl. f. Bakt. (Abt. 1)* **29**:231, 1901.
  - 167. Shaw, J. D.: *Brooklyn M. J.* **1**:14, 1888.
  - 168. Chassiotis: *Monatsh. f. prakt. Dermat.* **6**:1039, 1887.
  - 169. Looft, C.: *Virchows Arch. f. path. Anat.* **128**:215, 1892.
  - 170. Saingin: *Deutsche med. Wchnschr.* **24**:475, 1898.
  - 171. Jeanselme, E., and Marie, P.: *Rev. neurol.* **6**:751, 1898.
  - 172. Tschirjew, S.: *Arch. de physiol.* **6**:614, 1879.
  - 173. Pestana, C., and Bettencourt, A.: *Zentralbl. f. Bakt. (Abt. 1)* **19**:698, 1896.
  - 174. Sudakewitsch, J. J.: *Zentralbl. f. Chir.* **12**:567, 1885.
  - 175. Ermakóva, N.: *Internat. J. Leprosy* **4**:325, 1936.

## PROTOZOAN DISEASES

*Malaria.*—Malaria from the standpoint of prevalence is no doubt the most important of all tropical diseases. It is one of the most frequent of all infectious diseases. It is well known throughout the world and is widely distributed over all parts of the tropical and subtropical regions. Records of the League of Nations from 65 countries showed 17,750,000 persons treated for this disease in 1932 alone (Brennan<sup>176</sup>). In India, with a population of 353,000,000, about 100,000,000 cases occur annually. Malaria is an infectious disease due to a parasite transmitted to man by the bite of an infected mosquito and characterized clinically by periodic attacks of fever associated with anemia. The causative parasite is a protozoan which passes its asexual cycle in man, who is the intermediate host, and its sexual cycle in the Anopheles mosquito, which is the definitive host. There are three types of this disease. The most common type is the benign tertian type, caused by *Plasmodium vivax*. This form is the most widespread and is the form seen most frequently throughout the temperate zones. The quartan malaria is relatively infrequent and is caused by *Plasmodium malariae*. The estivoautumnal or malignant tertian malaria is caused by *Plasmodium falciparum* and is encountered chiefly in the badly infected districts of the warmer parts of the world. It is the prevailing form in India, China and Central Africa and also is by far the most dangerous form because of its tendency to produce pernicious and malignant manifestations, especially in regard to the central nervous system. In a recent bulletin of the United States Army Medical Department a series of 6,059 cases of malaria was reported in 140 of which, or 2.3 per cent, encephalitic malaria resulted (Fitz-Hugh and co-workers<sup>177</sup>).

The clinical picture in malaria affecting the brain is most variable, since almost any part of the organ may be involved, with concomitant symptoms. Often the clinical picture changes so rapidly that there is great overlapping of symptoms in a single case. It is for this reason that numerous types of cerebral malaria have been described, such as "meningeal type," "encephalic type," "cerebellar type," "myelitic type," "hemiplegic type" and so on. The onset of this disease varies within wide limits. Many of the patients are admitted to the hospital in coma, convulsions, delirium or merely lethargy; however, in most instances a careful history will reveal some premonitory symptoms during the preceding days consisting of intense headaches, nausea and vomiting, backache, nuchal pain, photophobia and vertigo. Generally the most common early complaints are intense headache, somnolence and disorientation.

176. Brennan, E. T.: M. J. Australia 1:189, 1944.

177. Fitz-Hugh, T., Jr.; Pepper, D. S., and Hopkins, H. U.: Bull. U. S. Army M. Dept., 1944, no. 83, p. 39.

Much has been written on the lesions occurring within the brain in the so-called cerebral form of malaria. In the latter part of the nineteenth century Marchiafava and Bignami,<sup>178</sup> Mannaberg<sup>179</sup> and McCallum<sup>180</sup> described the cerebral changes fairly accurately from the point of view of the pathologists in some excellent monographs. Since that time considerable additions have been added to knowledge of the genesis and the pathologic aspects of malaria.

Probably the best known lesions are the vascular and hemorrhagic disturbances. For years the petechiae were considered as the classic lesions (Gaskell and Millar<sup>181</sup>; Dürck<sup>182</sup>; Rigdon<sup>183</sup>). These hemorrhages are of both the ball and the ring type and appear predominantly in the white matter. Dürck in a review of 30 cases of death from acute cerebral malaria was able to find that hemorrhages had occurred in but 40 per cent and felt that the associated cerebral changes were often more widespread and more important. Usually the capillaries and the smaller blood vessels show the most severe changes (Ogurtsova<sup>184</sup>; Dürck<sup>185</sup>; Margulis<sup>186</sup>; Gaskell and Millar<sup>181</sup>; Lafora<sup>187</sup>). Their endothelial cells are frequently swollen and even proliferated, reducing or even obliterating a part or the entire vascular lumen. Many of these cells are filled with pigment, fat granules or parasites. They often swell and break off into the lumen, producing a thrombus. Many of the cerebral capillaries are filled with malarial parasites, parasitized red cells or red cells filled with granules of pigment. The number of such thrombosed vessels varies from case to case, and in some instances they are extremely difficult to observe in spite of extensive clinical symptoms.

Probably more common than the vascular hemorrhagic lesions are the focal perivascular areas of necrosis, gliosis or both. Numerous variations have been described for these perivascular lesions, but probably all are related and represent variations of the same process (Marinesco<sup>188</sup>; Margulis<sup>186</sup>; Dürck<sup>185</sup>; Gaskell and Millar<sup>181</sup>; Dhayagude

178. Marchiafava, E., and Bignami, A.: On Summer-Autumn Malarial Fever, translated by J. H. Thompson, Publication 150, London, New Sydenham Society, 1894, p. 1.

179. Mannaberg, J.: The Malarial Parasites, translated by R. W. Felkin, Publication 150, London, New Sydenham Society, 1894, p. 235.

180. McCallum, W. G.: *J. Exper. Med.* **3**:103, 1898.

181. Gaskell, J. F., and Millar, W. L.: *Quart. J. Med.* **13**:381, 1920.

182. Dürck, H.: *Arch. f. Schiffs- u. Tropen-Hyg.* **29**:43, 1925.

183. Rigdon, R. H.: *South. M. J.* **37**:687, 1944.

184. Ogurtsova, A. S.: *Nevropat. i psikhiat.* **9**:42, 1940.

185. Dürck, H.: *Münchén. med. Wehnschr.* **68**:33, 1921.

186. Margulis, M. S.: *Neurol. Centralbl.* **33**:1019, 1914.

187. Lafora, G. R.: *J. f. Psychol. u. Neurol.* **19**:209, 1912

188. Marinesco, M. G.: *Brain* **44**:223, 1921.

and Purandare<sup>189</sup>; Rigdon and Fletcher<sup>190</sup>). Margulis in 1914 first emphasized the presence of purely necrotic lesions in cerebral malaria. This type is invariably associated with a small occluded capillary containing pigment or parasitized red cells. The central necrosis reveals complete destruction of the brain tissue, which appears as an amorphous granular mass. Outward, toward the periphery of the lesion, there is less involvement, the tissues being fragmented and invaded by fat granule cells. These foci vary in number and in size, often measuring from 30 to 700 microns in diameter. Rigdon and Fletcher also described scattered areas of perivascular demyelination associated with these necrotic lesions.

When the purely necrotic type of lesion is surrounded by a peripheral zone of glial elements, it is usually called "Dürck's granuloma." In 1921 Dürck<sup>185</sup> emphasized the importance of this glial increase occurring particularly around the necrotic focus. He pointed out that the glial elements were frequently elongated and arranged radially about the necrotic focus. Red cells were occasionally observed around the glial elements. In the more chronic lesions the glial proliferation often extended inward to replace the necrotic tissue, resulting thus in a small patch of sclerosis.

Margulis<sup>186</sup> in 1914 was the first to describe glial nodules occurring in early malarial lesions entirely independent of necrosis of tissue. Similar lesions have been observed by Thomson and Annecke<sup>191</sup> and Marinesco.<sup>188</sup> In the cases of Thomson and Annecke these nodules were situated in the subcortical regions, while in Marinesco's case, they occurred chiefly in the gray matter and measured 210 to 130 microns in diameter.

A curious type of necrotic lesion was recently observed by Rigdon and Fletcher<sup>190</sup> in a case of cerebral malaria. They observed varying sized holes measuring 1.5 to 80 microns in diameter scattered throughout the white matter of the cerebrum and the cerebellum. Fragments of degenerating fibers were seen either free or extending across some of these spaces. These lesions they called "perforate lesions."

Damage of nerve cell in cerebral malaria was recognized as early as 1890 by Marchiafava and Bignami.<sup>178</sup> Lafora<sup>187</sup> in 1912 reviewed these neuronal alterations in detail. He described chromatolysis, swelling and vacuolation of the cytoplasm and degeneration of the medullated fibers. Since them, nerve cell changes have been observed by

189. Dhayagude, R. G., and Purandare, N. M.: Arch. Path. **36**:550, 1943.

190. Rigdon, R. H., and Fletcher, D. E.: Arch. Neurol. & Psychiat. **53**:191, 1945.

191. Thomson, J. G., and Annecke, S.: J. Trop. Med. **29**:343, 1926.

many investigators (Margulis<sup>186</sup>; Dürck<sup>185</sup>; Cerletti<sup>192</sup>; Rigdon and Fletcher<sup>190</sup>). The neuronal changes are most variable from case to case, often appearing in many scattered regions of the nervous system. Dürck<sup>185</sup> and Rigdon and Fletcher<sup>190</sup> described extensive degeneration and depletion of the Purkinje cells of the cerebellum. This localization of the changes to the cerebellum could well account for the occasional predominant cerebellar syndromes in this form of malaria. The nature of the nerve cell damage naturally varies with the severity and the chronicity of the involvement. Many of the cells reveal only mildly acute changes, while others reveal severe involvement, consisting of vacuolation, fragmentation and pyknosis. Invariably the damaged cells are observed scattered among intact elements, and in many areas the involved neurons predominate about the altered vessels. Dürck described extensive neuronophagia in some of his cases. Many of the damaged nerve cells evoked proliferation of glia, which ultimately invaded the damaged cells.

Actual inflammatory cellular reaction occurs infrequently in malaria. Gaskell and Millar<sup>181</sup> and Dürck<sup>182</sup> noted collections of lymphocytes around meningeal vessels.

A word might be said regarding present day views of the genesis of the cerebral lesions. By far the most popular and predominant view is that the cerebral lesions are vascular in origin and due to an embolic thrombotic process (Marchiafava and Bignami<sup>178</sup>; Dürck<sup>182</sup>; Dhayagude and Purandare<sup>189</sup>). Generally these investigators felt that the parasitized red cells, especially those with *P. falciparum*, had a tendency to agglutinate and adhere to the vessel wall. These agglutinated parasitized cells plus the swollen endothelial cells produced partial or complete vascular occlusion with resulting focal ischemic reactions. More recently various investigators, such as Gaskell and Millar<sup>181</sup> and Kean and Smith,<sup>193</sup> have questioned the thrombotic concept of these lesions. They were unable to find sufficient evidence of the so-called "cerebral plugging" to account for the severity of the cerebral symptoms. Manna-berg<sup>179</sup> as early as 1896 suggested that a toxin was liberated by the parasite which might account for the damage of the brain. The existence of such a toxin has been advocated by many investigators (Ewing<sup>194</sup>; Lafora<sup>187</sup>; Thomson and Annecke<sup>191</sup>). Most of these investigators based their opinions primarily on the fact that malarial coma occurs without parasites or pigment being found in the brain. However, the presence of such a toxin has never been demonstrated either *in vivo* or

192. Cerletti, U.: Histologische und histopathologische Arbeiten über die Grosshirnrinde, Jena, G. Fischer, 1910, vol. 4, p. 169.

193. Kean, B. H., and Smith, J. A.: Am. J. Trop. Med. **24**:317, 1944.

194. Ewing, J.: J. Exper. Med. **6**:119, 1902.

in vitro. More recently Rigdon<sup>183</sup> and Rigdon and Fletcher<sup>190</sup> have advocated the theory that anoxia is the basis for the cerebral lesions. These investigators argued that in severe malaria there is a rapid drop in hemoglobin and red cells resulting in severe anemia. The red cells surviving are often so heavily parasitized that their oxygen-carrying capacity is reduced. These two conditions, therefore, result in cerebral anoxia with the development of cerebral lesions, all of which can be reproduced in anoxic conditions of other etiology.

*Trypanosomiasis*.—The flagellate protozoa parasitic to man include those forms inhabiting the oral, intestinal and anal cavities, where they are but slightly pathogenic, and those living in the blood and the tissues, the hemoflagellates, which are highly pathogenic. Of the latter there are two groups, the trypanosomes, causing African and South American trypanosomiasis, and Leishmania forms, causing the various types of leishmaniasis. Both groups require invertebrate as well as vertebrate hosts to complete their life cycle, and both are more or less tropical in distribution.

(a) African Trypanosomiasis: African trypanosomiasis, or sleeping sickness, is caused by *Trypanosoma gambiense*, which is transmitted from man to man by the bite of species of tsetse flies. The infection is characterized by fever for a period, followed by an inflammatory reaction of the lymphatic system and by meningoencephalitis with symptoms of physical and mental lethargy. It occurs throughout equatorial Africa and is severe in some districts, being almost 100 per cent fatal if without treatment. Species of wild and domestic animals may act as a reservoir of the disease and in late years the incidence has been increasing in many areas.

Clinically the disease progresses from an early acute or incubation stage, lasting from a few months to several years, to a chronic cerebral or "sleeping sickness" stage with eventual fatal termination. The cerebral stage is characterized by the advent of various neurologic and psychiatric manifestations. Tremors, weakness, spasticity, and disturbances of personality become evident, and as the disease progresses the typical deterioration, emaciation and somnolence gradually increase until death intervenes. This stage begins early in the second year, occasionally sooner, and lasts from a few weeks to several months or even years. It is highly fatal. Death occurs from terminal meningitis, pneumonia or other secondary infections.

Although lesions of the central nervous system in sleeping sickness were described as early as 1840, the first adequate descriptions of the pathologic picture were that of Manson and Mott in 1900<sup>195</sup> and that

195. Manson, P., and Mott, F. W.: Tr. Path. Soc. London 51:99, 1900.

of Mott in 1906.<sup>196</sup> Mott characterized the disease as chronic adenitis followed by chronic inflammatory changes in the lymphatics of the brain and the spinal cord. These changes were manifested by proliferation and overgrowth of the neuroglia cells, especially of those related to the subarachnoid space and the perivascular lymph spaces. The lepto-meninges contained in addition numerous lymphocytes. The perivascular neuroglial increase was most marked in the molecular layer of the cortex and in the subcortical white matter. Enclosed in the neuroglial network were lymphocytes, proliferated endothelial cells and plasma cells. The relative abundance of plasma cells in the perivascular spaces was characteristic. In the more chronic cases, a large type of cell with a deep blue nucleus and eosinophilic granules in the cytoplasm was observed. Cells of this type were called "morula cells" and were felt to be degenerated plasma cells. Capillary hemorrhages were found in all stages of the disease. Ganglion cells showed chromatolysis with little change in their size or shape. There was some diffuse atrophy of the fiber tracts in the white matter. Mott postulated that the clinical symptoms were caused by the altered function of ganglion cells, due to ischemia and improper nutrition, and that the pathologic changes began with the entrance of trypanosomes into the cerebrospinal fluid. In a subsequent paper<sup>197</sup> he stressed the proliferation of endothelial cells and stated that the neuroglial composition of the perivascular infiltrate was much less extensive than he had previously indicated.

Similar observations were recorded by Warrington,<sup>198</sup> Low and Castellani<sup>199</sup> and Eisath.<sup>200</sup> Low and Castellani in addition found diffuse degenerative changes in the axis-cylinders and medullary sheaths in the spinal cord, and Eisath recorded glial cell enlargement and proliferation in the cord. Thomas and Breinl<sup>201</sup> reported a case in which capillary hemorrhages were extremely prominent, in places almost destroying the brain tissue. These petechiae were most marked in the pons and the spinal cord. Proliferation of the ependymal cells of the lateral ventricles was also seen. In another case there was considerable alteration in the large nerve cells of the brain and the cord with chroma-

196. Mott, F. W., in Reports of the Sleeping Sickness Commission, Royal Society of London, 1906, no. 7, vol. 15, p. 1.

197. Mott, F. W.: Proc. Roy. Soc. Med. 4:1, 1910.

198. Warrington, W. B.: Brit. M. J. 2:929, 1902.

199. Low, G. C., and Castellani, A., in Reports of the Sleeping Sickness Commission, Royal Society of London, 1903, no. 2, vol. 5, p. 14.

200. Eisath, G.: Arch. Path. 3:647, 1927.

201. Thomas, H. W., and Breinl, A.: Trypanosomes, Trypanosomiasis, and Sleeping Sickness: Pathology and Treatment, Memoir 16, Liverpool School of Tropical Medicine, 1905.

tolysis, nuclear changes and ghost cells. Stevenson<sup>202</sup> demonstrated trypanosomes scattered throughout the brain substance, especially in the frontal lobes, the pons and the medulla, and more numerous in the white matter than in the cortex. In addition to the typical perivascular infiltration, he noted some vessels with thickened walls and contracted lumens but with few perivascular elements. He expressed the opinion that the perivascular infiltration developed when the trypanosomes migrated from the vessels to extravascular tissue. Peruzzi<sup>203</sup> found a relative increase in the number of "morula cells" in the infiltrations around vessels within the brain substance. They were also present in large numbers in the white and gray matter in close connection with nerve cells. He thought that they were of neuroglial origin and that their presence was accompanied by severe changes in nerve cells. He stressed the involvement of the choroid plexus and suggested that the trypanosomes gained access to the ventricular fluid through the choroid plexus. Spielmeyer<sup>204</sup> was of the opinion that the perivascular neuroglial proliferation was non-specific and represented a reaction to inadequate cerebral nutrition resulting from the vasculitis. The cellular changes were most often seen in the deep layers of the gray matter with accompanying alterations of nerve fibers. He considered the "morula cells" to be degenerative and vascular, not neuroglial, in origin. Bertrand, Babet and Sicé<sup>205</sup> after an extensive review of the literature and a study of untreated patients, concluded that from the very first sleeping sickness was a diffuse type of meningoencephalitis with marked diffuse perivascular infiltration. The cellular elements of the infiltration included both neuroglial and vascular elements with prominence of plasma cells. The white matter was usually involved to a greater degree, and the amount of neuroglial proliferation was related to some extent to the chronicity of the infection. Ganglion cell changes were slight and acute in nature. De Savitsch and Freeman<sup>206</sup> considered the principal effect of cerebral trypanosomiasis to be a diffuse change in the myelinated fibers with infiltration and isomorphic gliosis. They felt that the somnolence and the symptoms of deterioration resulted from interruption

202. Stevenson, A. C.: Proc. Roy. Soc. Trop. Med. & Hyg. **16**:135 and 384, 1922.

203. Peruzzi, M.: Pathologico-Anatomical and Serological Observations on the Trypanosomiases, Final Report of the League of Nations International Commission on Human Trypanosomiasis, Geneva, Switzerland, 1927, vol. 3.

204. Spielmeyer, W.: Die Trypanosomenkrankheiten und ihre Beziehungen zu den syphilogenen Nervenkrankheiten, Jena, G. Fischer, 1908.

205. Bertrand, I.; Babet, J., and Sicé, A.: Ann. Inst. Pasteur **54**:91, 1935.

206. de Savitsch, E., and Freeman, W.: M. Ann. District of Columbia **8**:231, 1939.

of the association pathways. Only in the floor of the third ventricle were the infiltrations so extreme as to invade the neighboring cerebral tissue.

(b) South American Trypanosomiasis: South American trypanosomiasis, or Chagas' disease, was first described by Chagas<sup>207</sup> in Brazil in 1909. It is caused by the flagellate *Trypanosoma cruzi*, which is transmitted from the animal reservoir to man by a species of reduviid bugs. Acute and chronic forms of the disease are described. In the acute stage the parasites are found in the circulating blood and produce fever, edema, adenitis and convulsions, while in the chronic forms the symptoms are produced by the parasites as they become localized in various tissues of the body, especially the heart and the brain. The cases of this disease are scattered throughout South and Central America, but most of the reported cases occurred in Brazil and Argentina. It is not a common infection, and the cases are numbered only in the hundreds. Most of the patients have been infants and young children, and it is among this age group that the acute form with a high mortality rate occurs. Adults infected experimentally have suffered only mild symptoms, and in some the disease has been discovered only by routine examination of the blood.

Symptoms of involvement of the nervous system are particularly evident during the acute phase, and in many cases comprise the greater part of the clinical picture. Convulsions are most common and are usually present terminally, though they may occur initially or at any time during the disease. Many symptoms referable to the nervous system, such as mental retardation, apathy, speech disorders and idiocy, are described in chronic cases, but are most likely due to cretinism rather than to trypanosomiasis, though sequelae from severe infections might be quite similar. Paralysis, disorders of locomotion, incoordination, convulsions and coma have been noted in dogs experimentally infected.

The general pathology of Chagas' disease has been summarized by Yorke<sup>208</sup> as indicating degeneration of the invaded cells with accompanying cellular infiltration and eventual fibrosis of the affected tissues. Heart, brain and liver are most severely involved. Inside the cell the parasite has a form resembling *Leishmania*, being a small round or oval body with a nucleus and a kinetoplast. In this form it multiplies within the cell, forming intracellular cysts, which eventually rupture, liberating the parasites into the surrounding tissue. . . .

The involvement of the nervous system was first described completely from the point of view of the pathologist by Vianna.<sup>209</sup> He found inflam-

207: Chagas, C.: Mem. Inst. Oswaldo Cruz 8:5, 1916.

208. Yorke, W.: Trop. Dis. Bull. 34:275, 1937.

209. Vianna, C.: Mem. Inst. Oswaldo Cruz 3:276, 1911.

matory nodules in the gray and the white matter in all parts of the central nervous system, but most numerous in the basal nuclei, the pons and the spinal cord. They were of varying size and had no direct relationship to the neighboring vessels. They were frequently surrounded by a diffuse zone of leukocytic infiltration. The parasites were seen inside round mononuclear cells which, though difficult to recognize because of distortion, were thought to be neuroglia cells. Inflammatory changes were present in the meninges, and there was slight perivascular infiltration. Torres and Villaca<sup>210</sup> confirmed these observations and stated that the cells making up the inflammatory nodules were neuroglia and mononuclear cells.

Crowell<sup>211</sup> in studying an 8 month old infant who died during an acute infection found similar changes in the brain. The parasitized cells were few and were not seen near inflammatory foci such as those just described. De Coursey<sup>212</sup> found in addition to the inflammatory foci mild perivascular infiltration with round cells and occasional polymorphonuclear cells. The foci were composed of irregularly flame-shaped nests of small round cells with little cytoplasm. There were infrequent large cells whose distended cytoplasm contained 20 to 80 leishmaniform parasites. Lundeberg<sup>213</sup> described foci of microglia cells with round, oval or vesicular nuclei and pale cytoplasm. Some of the cells contained clusters of parasites, and in some places the parasites appeared to lie free in nerve fibers. Many small monocytes were scattered among the larger parasitized cells. The foci were frequently associated with small blood vessels, the latter usually exhibiting marked swelling and hyperplasia of the endothelium. A few degenerated nerve cells and an occasional capillary hemorrhage were noted.

Johnson and de Rivas<sup>214</sup> examined a 3 month old infant in whom the meninges were so edematous as to present the appearance of a gelatinous capsule covering the brain. A small amount of clear fluid expressed contained motile trypanosomes. Inflammatory foci of mesoglia cells and monocytes, some with areas of necrosis, were scattered throughout the nervous system, but parasites were found only in the cerebrum. Parasites were seen within the protoplasmic astrocytes, also, but no inflammatory reaction was observed around these cells.

Mazza and his associates<sup>215</sup> concluded that pathologic study provided no characteristic cytologic picture which would make one think

210. Torres, C. M., and Villaca, J.: Mem. Inst. Oswaldo Cruz **11**:80, 1919.

211. Crowell, B. C.: Am. J. Trop. Med. **3**:425, 1923.

212. de Coursey, E.: Am. J. Trop. Med. **15**:33, 1935.

213. Lundeberg, K. R.: Am. J. Trop. Med. **18**:185, 1938.

214. Johnson, G. M., and de Rivas, G. T.: Am. J. Trop. Med. **16**:47, 1936.

215. Mazza, S.; Friere, R. S., and Salica, P. N.: Investigaciones sobre la enfermedad de Chagas, Regional Argentina, University Buenos Aires, 1942.

of the disease. They found the meninges extensively and diffusely infiltrated by leukocytes which advanced along the perivascular spaces deep into the white substance of the brain. The infiltration extended outward into the circumscribed nodules, but its perivascular nature was still evident. The cellular elements consisted of macrophages, polymorphonuclear leukocytes and mononuclear cells. In addition proliferation of the glial elements occurred, and in the more severely involved areas degeneration of brain tissue. The distribution of parasites was roughly parallel to the intensity of the leukocytic reaction. Organisms were occasionally seen in glial cells, fat granule cells and macrophages or free from cells in nerve tissue or the perivascular space.

Most of the changes described by the foregoing authors were demonstrated experimentally in dogs by Villela and Torres.<sup>216</sup> Gross examinations yielded negative results. Microscopically the most prominent lesions were small inflammatory foci seen in the neighborhood of precapillary or capillary vessels in both the gray and the white matter in all parts of the central nervous system, though less often in the cerebellum. The structure of these foci varied with their age. In the first stages they were made up of macrophages, leukocytes and some plasma cells, generally in close relationship to the vessels. In the more advanced foci, the fat granule cells were more prominent, and there was evident disintegration of nerve tissue with formation of fissures and cavities. In the oldest foci, macrophages were no longer seen, and the cells present had become elongated to resemble fibrous neuroglia. The vascular lesions consisted of perivascular infiltrations of endothelial cells, lymphocytes and plasma cells. These cells first lodged in the adventitia and thence spread to the lymphatic spaces in the more intense reactions. The endothelial cells were always dominant; they rarely contained parasites. The vascular infiltration was never diffuse but limited to a single vessel or part of a vessel. The most commonly involved nerve cells were the Purkinje cells of the cerebellum and the pyramidal cells of the cerebral cortex. The lesions of these cells consisted of swelling and vacuolation or of shrinking and atrophy. Neuronophagia was common. Parasites were observed most often in the protoplasm of macrophages, in the fat granule cells and occasionally in the protoplasmic neuroglia. It appeared that the parasites pierced the capillary wall and were taken up by the neuroglial cells. The infected cells then became foci for the infection of other cells.

(c) Leishmaniasis: Leishmaniasis is caused by a protozoan parasite belonging to the genus *Leishmania*. It is made up of two forms, a visceral

216. Villela, E., and Torres, C. M.: Mem. Inst. Oswaldo Cruz **19**:198, 1926.

form commonly known as kala-azar and a cutaneous form known as tropical or oriental sore.

Kala-azar is an infectious disease caused by *Leishmania donovani* and probably spread by the sandfly. Although a tropical disease, it does not occur in the hot climates and is more prevalent in the cooler seasons. It is found in Africa, the Mediterranean area of Europe, and Asia, being extremely frequent in India, China and Manchuria. The parasite as seen in man is known as the Leishman-Donovan body. It is a round or oval unicellular organism measuring 2 to 5 microns in diameter and is found chiefly in the reticuloendothelial cell or the tissue macrophage. It multiplies within the cell of the host, replacing the entire cell, which ultimately becomes destroyed, liberating the parasites. Experimentally dogs, cats, mice, hamsters and monkeys can be infected with this disease. The dog may be the principal animal reservoir of the disease in certain localities.

The incubation period of kala-azar is from two to four months. The onset may be sudden or gradual, being ushered in by such symptoms as fever, chills, vertigo, headache, malaise, abdominal pain, nausea and vomiting. The temperature curve is irregular. There is progressive loss of weight with general wasting. Most characteristic of this disease is the enlargement of the spleen and the liver. Involvement of the nervous system is most unusual. The headaches are never severe, and mental symptoms rarely occur even when the fever is high.

In view of the infrequency with which this disease involves the central nervous system, reports on the pathologic changes within the brain are few and based mostly on study of animal tissues.

La Cava<sup>217</sup> in 1911 published studies of a fatal case of infantile leishmaniasis. The dura was covered with petechiae and the meninges were congested, but the brain seemed uninvolved. Similar findings have been reported by Christophers.<sup>218</sup>

Microscopically, parenchymatous lesions have been reputed not to occur in the brain of man. In experimental animals, parasites have not been found in the brain but have been found in the meninges. Meleney<sup>219</sup> studied material from 19 hamsters inoculated with *L. donovani*. Parasitized cells were observed only in the meninges and the choroid plexus, usually in the loose tissue just outside the blood vessels. Nicolau and

217. LaCava, F.: Atti Roy. Acad. Lincei R. C. **20**:778, 1911.

218. Christophers, S. R.: On a Parasite Found in Persons Suffering from Enlargement of the Spleen in India, Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, Calcutta, Supt. Gov. Print., 1904, no. 11.

219. Meleney, H. E.: Am. J. Path. **1**:147, 1925.

Perard<sup>220</sup> observed similar localizations of canine leishmaniasis. They surmised that the choroid plexus arrested the organisms and prevented them from reaching the brain. In only 1 dog did they find an intracerebral lesion; this was in the region of the floor of the fourth ventricle. There was an intense parenchymatous reaction with infiltrations of mononuclears, polymorphonuclears and plasma cells. An occasional degenerated parasite could be seen in the cellular reaction. Hoeppli<sup>221</sup> observed some neuronophagia and degeneration of ganglion cells in hamsters experimentally infected with *L. donovani* and *L. tropica*. Even when a suspension of Leishman-Donovan bodies was inoculated intracerebrally in Chinese hamsters, focal parenchymatous lesions failed to develop.

*Relapsing Fever.*—Relapsing fever is a febrile spirochetal infection which is widely distributed in many parts of the world and is transmitted to man by the tick and the body louse. There are many different species of spirochetes producing relapsing fever. Some of the more common species are: *S. recurrentis* (European), *S. duttoni* (Central African), *S. novyi* (American), *S. berbera* (North African). In general the European, Indian, Chinese and West African infections are transmitted from man to man by body lice, while the Central African and American forms are transmitted by ticks. In Africa, relapsing fever ranks next to malaria in frequency and is found chiefly in Egypt, Algeria and West Africa. Relapsing fever has appeared recently in endemic foci in many states within the United States. From Texas alone 258 cases occurring between 1930 and 1935 were reported by Kemp and his associates,<sup>222</sup> while 138 cases were studied in California between 1930 and 1938 by Wheeler.<sup>223</sup>

Clinically there is an acute onset of fever, malaise and headaches. The temperature rises rapidly and remains elevated for a number of days, falling by crisis. After an afebrile period of four to eight days there is a similar but usually milder febrile episode. In the European type there are usually two to three paroxysms, while in the African type there may be as many as ten relapses. Neurologic complications are most frequent in the tick-borne infections and usually occur late. The frequency of cerebral involvement is difficult to compute and has varied from 4.5 per cent of cases (Adler and co-workers<sup>224</sup>) to 20 per cent (Cooper<sup>225</sup>;

220. Nicolau, S., and Perard, C.: Ann. Inst. Pasteur **57**:463, 1936.

221. Hoeppli, R.: Arch. f. Schiffs- u. Tropen-Hyg. **33**:101, 1929.

222. Kemp, H. A.; Moursund, W. H., and Wright, H. E.: Am. J. Trop. Med. **13**:425, 1933.

223. Wheeler, C. M.: Am. J. Trop. Med. **18**:431, 641, 1938.

224. Adler, S.; Theodor, V., and Schrieber, H.: Ann. Trop. Med. **31**:25, 1937.

225. Cooper, E. L.: M. J. Australia **1**:635, 1942.

Scott<sup>226</sup>). Experimentally it has been recognized for years that the spirochete of relapsing fever is specifically neurotropic (Velu and co-workers<sup>227</sup>; Mathis and Durieux<sup>228</sup>; Levaditi and Anderson<sup>229</sup>; Lagrange<sup>230</sup>). Heronimus<sup>231</sup> thought that the brain was probably always infected in this disease. Plant<sup>232</sup> showed that the nervous system of the rabbit, which has natural immunity to spirochetal infection, does not have such immunity to infection with *S. duttoni* and can be infected while other organs remain free.

Clinically the most common neurologic complaint is intense headache, often occipital in distribution. The headache recurs with each attack of pyrexia and often persists for weeks after the temperature subsides (Cooper<sup>225</sup>). The headache is usually accompanied by vertigo, insomnia and, in severe forms, by delirium. Probably the most frequent involvement in relapsing fever is that of the meninges (Liegeois and co-workers<sup>233</sup>; Lafforgue<sup>234</sup>; Scott<sup>226</sup>; Cooper<sup>225</sup>; Stones<sup>235</sup>). Cooper reported that one fifth of his patients had meningeal symptoms. Symptoms of encephalitic involvement have also been reported (Vialatte<sup>236</sup>; Liegeois and co-workers<sup>233</sup>; Scott<sup>226</sup>).

Pathologic changes within the nervous system in relapsing fever are not too numerous in spite of the neurotropism of the organism. Belezky and Umanskaja<sup>237</sup> reported the most complete study of the encephalic changes in man, based on 8 fatal cases. The leptomeninges were hyperemic, and the vessels were surrounded by monocytes, lymphocytes, plasma cells and a few red cells. Spirochetes were observed free in the subarachnoid space as well as within the pial veins. The cerebral cortex showed extensive degeneration of ganglion cells. Many of the neurons were acutely swollen and showed complete chromatolysis and some vacuolation. There was mild glial increase. Degenerated spirochetes were present throughout the second and the fourth cortical layers,

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- 226. Scott, R. B.: *Lancet* **2**:436, 1944.
  - 227. Velu, H.; Balozet, L., and Zoltner, G.: *Compt. rend. Soc. de biol.* **106**: 1089, 1931.
  - 228. Mathis, C., and Durieux, C.: *Bull. Soc. path. exot.* **23**:862, 1930.
  - 229. Levaditi, C., and Anderson, T. E.: *Compt. rend. Soc. de biol.* **100**:1121, 1929.
  - 230. Lagrange, E.: *Bull. Soc. path. exot.* **24**:804, 1931.
  - 231. Heronimus, E. S.: *Zentralbl. f. Bakt. (Abt. 1)* **105**:394, 1928.
  - 232. Plant, F.: *München. med. Wchnschr.* **73**:1552, 1926.
  - 233. Liegeois, R.; Pages, R.; Duguet, J., and Pouhin: *Presse méd.* **46**:531, 1938.
  - 234. Lafforgue: *Rev. de méd., Paris* **28**:916, 1908.
  - 235. Stones, R. Y.: *Kenya M. J.* **3**:27, 1926.
  - 236. Vialatte, C.: *Arch. Inst. Pasteur d'Algérie* **4**:56, 1926.
  - 237. Belezky, W. K., and Umanskaja, R. M.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **129**:21, 1930.

unrelated to the vessels. Some spirochetes were found in the glial cells but none in the white matter. The basal ganglia showed acute swelling of the cells. Spirochetes were present in the floor of the fourth ventricle between the ganglion cells of the olive. Jahnel and Lucksch<sup>238</sup> made sections of the brains of 2 soldiers who died of relapsing fever and found spirochetes in both. These were observed chiefly in the vessels, although a few were lying free between the cortical neurons. There were some petechiae in the pia.

Levaditi and co-workers<sup>239</sup> described in detail the changes in the brains of rabbits and monkeys infected with relapsing fever. These resembled closely the changes observed in man. The meninges contained many perivascular and diffuse accumulations of monocytes. The most severe encephalic changes occurred in the cortex, in the white matter along the walls of the lateral ventricles, and within the midbrain and the medulla. The changes were chiefly parenchymatous and vascular. The former consisted of foci of mononuclears surrounding many vessels and extending outward to involve the neurons and the neuroglial elements. The perivascular accumulations were tremendous, often being ten to twenty layers deep. The vessels frequently showed endothelial increase with narrowing of the lumen. Many of the nerve cells showed chromatolysis and vacuolation.

*Rat Bite Fever.*—Rat bite fever is an acute infectious disease caused by Spirochaeta morsus muris, transmitted usually in the bite of a rat, less commonly in the bite of a ferret, a cat, a weasel, a dog or a squirrel (Ripley and Van Sant<sup>240</sup>). The disease is characterized by paroxysms of fever, an inflammatory reaction at the site of the bite, tenderness in the regional glands, an exanthem and various, often marked neurologic symptoms.

This disease has been recognized in India for many centuries and is very common in the Orient. It is endemic in Japan, where the mortality rate in untreated patients is 10 per cent. Within the last twenty years cases have been reported from Spain, Italy, France, Germany, Australia and America. According to Brown and Nunemaker,<sup>241</sup> 125 cases proved to be cases of rat bite fever were reported in the United States through 1940. In these there were no deaths.

In 1914 Schottmüller<sup>242</sup> described a case of rat bite fever from which a "streptothrix" was isolated. Similar organisms have been

238. Jahnel, F., and Lucksch, F.: Med. Klin. **23**:2003, 1927.

239. Levaditi, C.; Anderson, T. E.; Selbie, F. R., and Schoen, R.: Bull. Acad. de méd., Paris **103**:673, 1930.

240. Ripley, H. S., and Van Sant, H. M.: J. A. M. A. **102**:1917, 1934.

241. Brown, T., and Nunemaker, J. C.: Bull. Johns Hopkins Hosp. **70**:201, 1942.

242. Schottmüller, H.: Dermat. Wchnschr. **58**:77, 1914.

isolated from other cases of rat bite fever and the type has been called *Streptobacillus multiformes* (Lemierre and associates<sup>243</sup>; Place, Sutton and Willner<sup>244</sup>). The rat bite fever caused by this type of organism is clinically more benign and when compared with the form generally accepted in the foreign literature as caused by *Spirillum minus* (*Spirochaeta morsus muris*) is found somewhat dissimilar. In this study the true spirillum disease is referred to.

For a short time rat bite fever, because of its relapsing nature and the complete arsenical control of it, was used for the treatment of the meningoencephalitic type of psychosis with syphilis of the central nervous system (general paresis). Solomon and co-workers<sup>245</sup> instituted this treatment in 1926 when he inoculated 12 patients suffering from this condition. Since then 104 cases of rat bite fever have been artificially produced by inoculation of *Spirochaeta morsus muris* (Bayne-Jones<sup>246</sup>; Hershfield and co-workers<sup>247</sup>; Teitelbaum<sup>248</sup>). However, this form of treatment has been abandoned because of the 10 per cent mortality with some strains of this organism and because of the high incidence of complications (arthritis, myositis, convulsions and others).

Neuropsychiatric symptoms comprise some of the most common complaints in this illness and invariably appear at the height of the first few paroxysms of temperature. Patients with the most severe form complain of headache, vertigo, tinnitus, nausea and vomiting, and blurring of vision (Arkin<sup>249</sup>; Gilkey and Dennie<sup>250</sup>; McDermott<sup>251</sup>). Generally these symptoms subside with the fall of temperature. However, in some of the more severe infections the neurologic involvement is more extensive and more lasting. There may be lesions of cranial nerves, producing dysphagia, aphonia, amaurosis, deafness and papilledema (Ebert and Hesse<sup>252</sup>; Rasdolsky<sup>253</sup>). Motor disturbances are often quite definite and in certain patients may persist beyond the paroxysm as a permanent sequel. These motor symptoms consist of muscular twitchings, convul-

243. Lemierre, A.; Reilly, J.; LaPorte; A., and Morin, M.: Bull. Acad. de méd., Paris **117**:705, 1937.

244. Place, E. H.; Sutton, L. E., and Willner, O.: Boston M. & S. J. **194**:285, 1926.

245. Solomon, H. C.; Berk, A.; Theiler, M., and Clay, C. L.: Arch. Int. Med. **38**:391, 1926.

246. Bayne-Jones, S.: Internat. Clin. **3**:235, 1931.

247. Hershfield, A. S.; Kibler, O. A.; Colby, S.; Koenig, M. T.; Schmid, O. W., and Saunders, A. M.: J. A. M. A. **92**:772, 1929.

248. Teitelbaum, A. D.: M. Bull. Vet. Admin. **6**:263, 1930.

249. Arkin, A.: Arch. Int. Med. **25**:94, 1920.

250. Gilkey, H. M., and Dennie, C. C.: South. M. J. **32**:1109, 1939.

251. McDermott, E. N.: Quart. J. Med. **21**:433, 1928.

252. Ebert, B., and Hesse, E.: Arch. f. klin. Chir. **136**:69, 1925.

253. Rasdolsky, I.: Ztschr. f. d. ges. Neurol. u. Psychiat. **90**:188, 1924.

sions, hemiplegia, paresis and even muscular atrophies. Sensory involvement usually occurs in the most severe type of the disease and varies from paresthesias to areas of complete anesthesia, particularly in the extremities.

In spite of the fact that cerebral involvement seems to be very frequent in this disease, reports of autopsy studies on the central nervous system are greatly lacking in the literature. Miyaki<sup>254</sup> noted increase of spinal fluid and congestion of the vessels of the meninges and the cord. No histologic studies have been reported. Kaneko and Okuda<sup>255</sup> reported autopsy studies on a 70 year old man dying from rat bite fever. The age of this patient makes the histologic observations of questionable significance. The brain appeared hyperemic. Microscopically the neurons within both the brain and the cord showed definite swelling and fragmentation. Scattered cells were pyknotic. The nerve fibers revealed slight swelling and disintegration. Dragisic and Kaludjerski<sup>256</sup> reported a fatal case of rat bite fever. The patient, a 14 month old child, presented unilateral convulsive seizures. A spirillum was isolated from the blood. Grossly, a yellowish softened area filled with petechiae was observed in the genu of the corpus callosum. The cerebral tissues were hyperemic. Microscopically the involved area of the brain revealed numerous small foci of glia cells and perivascular erythrocytes. The softened area within the corpus callosum was filled with necrotic tissue and blood.

*Amebic Dysentery*.—Amebiasis is considered and classified as a tropical disease even though it occurs throughout the world and is extremely common in the temperate zones. The general idea prevails that "amebic dysentery" and "amebiasis" are synonymous terms and that amebic infections are uncommon except in the tropics. According to Craig,<sup>257</sup> 5 to 10 per cent of all the inhabitants of the United States are infected with *Endamoeba histolytica*.

*E. histolytica* primarily infects the intestine, reaching the bowel in drinking water or food which have been contaminated with fecal material containing amebic cysts. From the intestinal lesions the amebas may metastasize through the portal vein to the liver and rarely to other organs, such as the brain, where they set up a primary purulent encephalitis or an abscess of the brain. It has been estimated that hepatic abscesses occur in from 33 to 51 per cent of persons with amebic dysentery coming to autopsy, while only 3 per cent of these show

254. Miyaki, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **5**:231, 1899.

255. Kaneko, R., and Okuda, J.: J. Exper. Med. **26**:363, 1917.

256. Dragisic, B., and Kaludjerski, S.: Arch. f. Kinderh. **112**:168, 1937.

257. Craig, C. F.: J. A. M. A. **77**:827, 1921.

associated involvement of the brain (Clark<sup>258</sup>; Gehlen<sup>259</sup>; Kartulis<sup>260</sup>). About 65 cases of amebic abscess of the brain have been reported in the literature. In most of the cases there was also an associated abscess of the lung or the liver. In only 5 cases was the involvement of the brain an isolated finding (Kartulis<sup>260</sup>; Putney and Baker<sup>261</sup>; Stein and Kazan<sup>262</sup>).

When the brain is invaded by the endameba, there occur the symptoms and signs of diffuse suppurative meningo-encephalitis with the focal signs of an abscess. Generally the cerebral symptoms are preceded by signs of hepatic or thoracic involvement, often following operations for such complications.

The histologic changes occurring in amebic infections of the brain will naturally vary with the nature and the age of the infective process (Halpert and Ashley<sup>263</sup>; Kartulis<sup>260</sup>; Stein and Kazan<sup>262</sup>). Early lesions will reveal vascular congestion and thrombosis with secondary softening of tissue. Leukocytes, red cells and many amebas fill the softened area, which disintegrates to form a cavity filled with necrotic debris and surrounded by a rough, shaggy wall. In the smaller focal lesion this cavity becomes surrounded by a thick capsule composed of connective tissue, glial elements, blood vessels and mononuclear cells. The inner wall of the capsule often is composed of fat granule cells which have phagocytosed much of the necrotic brain tissue. Amebas often can be found in the abscess wall, appearing as hyaline masses or resembling compound granular cells, although they seem to be larger and possess a condensation of the outer cytoplasmic wall giving the cell a ringlike appearance. According to Stein and Kazan,<sup>262</sup> the amebas stain best with Best's glycogen stain, although they can be seen clearly with hematoxylin-eosin stain. The thickness of the abscess wall depends on the chronicity of the process. The small lesion may be practically replaced by the proliferative changes, while the larger one reveals a large purulent center filled with yellowish-brownish gelatinous pus and usually well walled off by a heavy capsule.

In the more extensive lesions, large areas of brain tissue become implicated, so that extensive softening occurs, surrounded by a wide zone of cerebral edema (Stein and Kazan<sup>262</sup>). This entire involved region reveals rarefied brain tissue filled with atrophic cellular elements and invaded by collections of mononuclear cells, fat granule cells and

258. Clark, H. C.: Am. J. Trop. Med. 5:157, 1925.

259. Gehlen, J. N.: Minnesota Med. 17:18, 1934.

260. Kartulis: Zentralbl. f. Bakt. (Abt. 1) 37:527, 1904.

261. Putney, F. J., and Baker, D. C.: Dis. of Chest 4:20, 1938.

262. Stein, A., and Kazan, A.: J. Neuropath. & Exper. Neurol. 1:32, 1942.

263. Halpert, B., and Ashley, J. D.: Arch. Path. 38:112, 1944.

extravasated red cells. Many amebas can be found within the softened brain tissue. Scattered in this area of more diffuse involvement are often seen smaller foci of actual necrosis of tissue with liquefaction and true abscess formation, so that numerous tiny abscesses can be identified throughout the involved region. Many of these small abscesses are surrounded by capsules of varying thickness. The small abscess is composed of a necrotic center containing leukocytes and red cells in varying stages of disintegration. Around the disintegrated center is a layer of fat granule cells, surrounded in turn by a firm layer of granulation tissue. Amebas are observed chiefly in the less severely involved tissue; that is, they are in the abscess walls, in the foci of rarefaction of tissue or within the meninges.

The meninges when implicated are thickened, containing aggregates of lymphocytes, polyblasts and fibroblasts.

Alexander and Wu<sup>264</sup> in studying a case of chronic amebic dysentery reported nonspecific and nonsuppurative changes resembling those seen in bacillary dysentery. They observed ischemic foci throughout the cerebral cortex, often replaced by glial scars. There were lipoid deposits in many of the cortical neurons. In the cerebellar cortex some of the Purkinje cells had disappeared and were replaced by glial shrubs. With silver stains the nerve cells revealed crumbling and destruction of the intracellular neurofibrils associated with argentophilia of the nuclei and of some of the glial cells.

#### ANIMAL PARASITES (HELMINTHS)

*Ascariasis*.—Ascariasis is caused by infection with the roundworm *Ascaris lumbricoides*, the helminth most commonly parasitic in man. It is worldwide in its distribution but is more prevalent and severe in the tropics owing to environmental conditions and to insanitary practices.

A number of cases are recorded in which symptoms suggesting meningeal and cerebral involvement have been ascribed to the toxic effects of the worms. Kollman<sup>265</sup> reviewed the literature and recorded cases in which meningitis, epileptiform convulsions, choreiform movements with delirium and paralysis of the lower extremities were symptoms which cleared up immediately on passage of the ascarids. Langhans<sup>266</sup> and Steber<sup>267</sup> described cases in which marked restlessness and delirium cleared up with removal of the worms. Cases simulating those of

264. Alexander and Wu, footnotes 109 and 112.

265. Kollman, A.: Arch. f. Kinderh. **82**:150, 1927.

266. Langhans, G. L.: Ztschr. f. Kinderh. **39**:344, 1925.

267. Steber: Deutsche med. Wchnschr. **43**:1040, 1917.

meningitis have been described by Turcan,<sup>268</sup> Valerio,<sup>269</sup> Ferrari<sup>270</sup> and Spieth.<sup>271</sup>

It is not clear whether these symptoms are produced by toxins elaborated by the living worms or by toxins released after their death in the intestine. Rachmannow<sup>272</sup> injected extracts of the worms into guinea pigs and found lesions of the central nervous system only in those animals which displayed clinical signs of involvement of the nervous system. The lesions he described were various degrees of chromatolysis of ganglion cells, nuclear changes with pyknosis and destruction of neurofibrillae. Neuroglial changes consisted of the appearance of ameboid cells and satellitosis. In addition there were patchy areas of demyelination in the white matter.

Cerebral symptoms may occasionally result when the larvae accidentally reach the general circulation and the brain. Fülleborn<sup>273</sup> found larvae in the brain a few days after feeding eggs of Ascaris to experimental animals, but he never noted cerebral symptoms even if the larvae were injected into the carotid artery. He surmised that most of the larvae passed through the brain back to the lungs and that hemorrhages occurred only rarely in the relatively firm brain tissue. Hoeppli<sup>274</sup> after feeding eggs to guinea pigs found larvae in the ventricle, starting to bore into the brain tissue. There was no surrounding reaction. In an animal killed six weeks after it had ingested eggs, he found a fully grown larva in a capillary in the brain stem, with no surrounding tissue changes. Yamaguchi<sup>275</sup> found larvae in the brain substance, less frequently in small arteries, and in the lateral ventricle and the choroid plexus in his animals. Occasionally he saw the moulted skins of larvae in the brain tissue. Meningeal and perivascular hemorrhages were fairly frequent, but little bleeding was noted within the brain tissue itself. The hemorrhages were most abundant in the cerebellum and the floor of the fourth ventricle. He also found a few nodules of proliferative glial cells scattered throughout the brain.

*Hookworm Infection.*—Hookworm infection (*ancylostomiasis*) is most prevalent in the tropical and subtropical countries of the world. The two most common species of worms causing the disease are *Necator americanus*, found in the New World and in equatorial Africa, and *Ancylostoma duodenale*, found in the Mediterranean regions and Asia

268. Turcan, H.: *Presse méd.* **30**:844, 1922.

269. Valerio, A.: *Brasil-med.* **40**:309, 1926.

270. Ferrari, G.: *Gior. di clin. med.* **14**:745, 1933.

271. Spieth, H.: *Virchows Arch. f. path. Anat.* **215**:117, 1914.

272. Rachmannow, A.: *Ann. Inst. Pasteur* **28**:181, 1914.

273. Fülleborn, F.: *J. Helminthol.* **7**:15, 1929.

274. Hoeppli, R.: *Virchows Arch. f. path. Anat.* **244**:159, 1923.

275. Yamaguchi, S.: *Arch. f. Schiffs- u. Tropen-Hyg.* **29**:589, 1925.

Minor. Both species are prevalent throughout the Orient. An infection rate of over 50 per cent prevails in most areas.

The symptoms referable to the nervous system are prominent. Sandwith<sup>276</sup> in 1894 stressed the symptoms of lethargy, eating dirt (geophagia), impotence, stupidity and decrease of the patellar reflexes. Ashford and King<sup>277</sup> noted headache, lack of attention to details and slight dulling of the mental faculties in patients with mild infections. In those whose disease was moderate the appetite was more exaggerated and dizziness, frequent headache, tingling of the feet, diminution of the patellar reflexes, passivity and mental depression were found. In those with severe disease there were dizziness, tinnitus, insomnia, absence of the patellar reflexes, impotence and amenorrhea, dulling of the intellect, depression, morbid parasthesias, blurred vision and extreme weakness.

The only suggestion that any pathologic changes take place in the nervous system with hookworm disease has been provided by Fülleborn.<sup>278</sup> He injected larvae into the jugular vein of the experimental animal and succeeded in recovering larvae from the brain. In an animal killed twenty-five minutes after the injection, two living larvae were found in the brain; in a second animal killed six and a half hours after the injection, five larvae were recovered and a small hemorrhage was seen in the brain tissue. In view of the path which the migrating larvae follow, it is possible that some larvae might reach the brain through the general circulation and cause symptoms.

*Strongyloidiasis*:—*Strongyloides stercoralis* has a life history somewhat similar to that of hookworm and produces a similar pathologic picture. It is capable of living outside the body indefinitely, and auto-infection may take place. The chief symptoms, according to Hinman,<sup>279</sup> are vague abdominal pains, diarrhea sometimes alternating with constipation, and loss of weight. The variability of the symptom complex is striking.

Faust<sup>280</sup> made a pathologic study of the infection in a series of animals. Four of 62 dogs were killed between eight and twenty days after exposure, because of symptoms indicating damage of the nervous system. One of these animals was in a state of tetany with spasticity of the left extremities and of the right side of the face. Incontinence subsequently developed. Similar symptoms were found in 2 additional dogs, and in the fourth dog a syndrome suggesting rabies developed on the eighth day. Microscopic examination of brain tissue showed scattered capillary hemorrhages, but no larvae were found. Yama-

276. Sandwith, F. M.: Lancet 1:1362, 1894.

277. Ashford, B. K., and King, W. W.: J. A. M. A. 49:471, 1907.

278. Fülleborn, F.: Arch. f. Schiffs- u. Tropen-Hyg. 30:679, 1926.

279. Hinman, E. H.: Rev. Gastroenterol. 5:24, 1938.

280. Faust, E. C.: Arch. Path. 19:769, 1935.

guchi<sup>275</sup> reported more severe but similar findings. Hemorrhages were numerous in the meningeal and the perivascular tissues, and they were more severe in the cerebellum. The areas most involved in the cerebrum were the cortex and the white matter, and especially the floor of the fourth ventricle. Larvae were seen free in the brain, in the small vessels and in the ventricles and the choroid plexus. A few proliferative glial nodules were seen scattered throughout the brain.

*Filariasis*.—The filarial worms are slender nematodes parasitic in the lymphatic and circulatory systems and the deeper tissues of the body. The adult females produce young prelarval worms known as microfilarias, which circulate in the blood and the lymph stream of the host. The life cycle is completed in an arthropod vector, and the worms are transmitted from man to man by various species of mosquitoes and biting flies.

Infection with *Wuchereria bancrofti*, commonly called filariasis, is the most widespread of all the filarial infections. In this disease the adult worms are found chiefly in the lymphatics. The incubation period is about a year, and the infection may remain entirely asymptomatic or may be evidenced by recurring attacks of lymphangitis. The chronic stages occur through lymphatic obstruction, with dilated lymphatics, enlarged lymph glands, lymphoceles, hydrocele and elephantiasis resulting. The circulating microfilarias apparently produce no symptoms.

Several investigators have thought, however, that the symptoms of involvement of the nervous system occasionally seen in filariasis are due to the presence of microfilarias in the brain. Mya<sup>281</sup> described the case of a patient admitted to the hospital for sudden loss of consciousness four hours previously. The next day the patient was semi-conscious. Right hemiplegia developed and the patient died sometime later. The only positive finding was the presence of microfilarias in the spinal fluid. Manson<sup>282</sup> stated that microfilarias can be found in the brain, and Anderson<sup>283</sup> reported a case in which numerous microfilarias were observed in the cerebral and cerebellar blood vessels. Some leukocytic infiltration was present around cerebral capillaries. Rodenwaldt<sup>284</sup> also found microfilarias in the cerebral capillaries, but he was unable to find any associated tissue changes. Wail, Popon and Prjadko<sup>285</sup> injected microfilarias into crows and observed marked damage of ganglion cells in various areas, with chromatolysis, pyknosis, lipoid

281. Mya, T.: Indian M. Gaz. **63**:636, 1928.

282. Manson, P.: Tropical Diseases, Paris. C. Naud, 1904.

283. Anderson, J.: Clinical, Pathological and Therapeutic Investigations (Filariasis in British Guiana), Research Memoir 7, London School of Tropical Medicine, 1924, vol. 5.

284. Rodenwaldt, E.: Arch. f. Schiffs- u. Tropen-Hyg. **10**:389, 1908.

285. Wail, S. S.; Popon, P., and Prjadko, F.: Virchows Arch. f. path. Anat. **259**:642, 1926.

degeneration, fragmentation of fibrillae and disappearance of cell outlines. Glial nodules surrounded the destroyed ganglion cells. Microfilarias were seen to fill the capillaries but not to cause obstruction. They stressed the need for investigation of human material.

Filariasis due to *Dipetalonema perstans* is characterized by absence of consistent symptoms. The adult worms prefer the serous cavities and the mesentery of the body, and the microfilarias are found more often in the larger vessels than in the peripheral blood. Most writers have thought that this parasite is nonpathogenic, while others have stated that it may produce symptoms similar to those seen in *Wuchereria* infection. Külz<sup>286</sup> described a case in which the patient had typical psychic and motor symptoms of sleeping sickness, but the spinal fluid contained only microfilarias. Chambon<sup>287</sup> reported on a case in which the cerebro-spinal fluid showed both microfilarias and trypanosomes. He surmised that the meninges were altered by the trypanosomal infection, permitting the entrance of the microfilarias. Rodhain<sup>288</sup> noted microfilarias in the choroid plexus in 1 case.

Loiasis, the form of filariasis caused by *Loa loa*, is distinguished by transient swellings and inflammatory phenomena in the subcutaneous tissues of the body, including the eye. It is possible in this infection as in the others for the microfilarias to lodge in the brain and cause symptoms.

The fourth filarial disease, onchocerciasis, is characterized by subcutaneous nodule formation and by absence of circulating microfilarias. The symptoms are due to the nodules and include local discomfort, inflammatory changes and erysipelas, and ocular disturbances. Robles<sup>289</sup> found among 500 patients 4 in whom the nodules had eroded both tables of the skull, with one nodule resting directly on the meninges. One patient had epileptiform seizures from irritation caused by a nodule which had penetrated the cranium. The microfilarias can apparently give rise to symptoms in this disease as in the others. Mira<sup>290</sup> found microfilarias in the optic nerve following enucleation of an eye for extensive ocular onchocerciasis, and Rodhain<sup>288</sup> observed them in the meninges in the case which he reported.

*Schistosomiasis*.—Schistosomiasis includes the group of diseases caused by species of flukes which inhabit the venous system of man in various tropical and subtropical countries. The three common species are *Schistosoma haematobium*, *Schistosoma mansoni* and *Schistosoma japonicum*. The first, *S. haematobium*, is endemic throughout Africa

286. Külz: Arch. f. Schiffs- u. Tropen-Hyg. **12**:547, 1908.

287. Chambon, M.: Bull. Soc. path. exot. **26**:613, 1933.

288. Rodhain, J.: Tr. Roy. Soc. Trop. Med. & Hyg. **30**:501, 1936.

289. Robles, R.: Bull. Soc. path. exot. **12**:442, 1919.

290. Mira, M. G.: Riforma med. **50**:858, 1934.

and the Near East. It usually localizes in veins of the pelvic plexus, particularly in the wall of the bladder, where the extruded ova cause symptoms of hematuria, dysuria, cystitis and other urinary difficulties. *S. mansoni* is found in South America and the Antilles in addition to the valley of the Nile River and other parts of Africa. It commonly localizes in the portal system and gives rise to chronic dysentery and various intestinal symptoms. Ova are found in great numbers in the liver, causing cirrhosis with splenomegaly. *S. japonicum* is found in the Far East, in China, Japan, Formosa, Celebes and the Philippines. It localizes in the portal system as does *S. mansoni*, and similar symptoms are produced, though they are often of a more severe nature.

The infection is a chronic one, and parasites may live in the body for many years. In some areas, the disease is severe, and a large percentage of the population is infected. Persons exposed to repeated infections are most likely to have chronic sequelae.

There are two ways in which the central nervous system is affected in schistosomiasis, according to Greenfield and Pritchard.<sup>291</sup> In one lesions are caused by the circulating ova which get past the barrier of the liver and the lung and act as emboli in the brain. In the other, the circulating larvae develop into adults in veins of the brain instead of in the portal system, and ova are extruded *in situ*. Though no adult worms have been found in such an aberrant location, this possibility was suggested to the authors on the basis of the large numbers of eggs circumscribed within the lesions which they observed. In the first of 2 cases which they reported the symptoms consisted of epileptiform seizures, hemianopia, scotomas, aphasia, clumsiness of the right hand and difficulty in thinking. The findings included injected optic disks, slight weakness of the right side of the face, increased reflexes on the right side, and a spinal fluid with 79 cells—93 per cent lymphocytes. A tumor was removed from just beneath the surface of the brain in the left parietal region, which proved to be a granuloma containing eggs of *S. japonicum*. In their second case the symptoms were persistent headaches and vomiting, scotomas, a field defect and difficulty of speech and finer movements of the right hand. A large tumor was similarly removed from the left parieto-occipital region. The pathologic observations were the same in both cases. Grossly the tumors were yellowish and friable. Cut sections revealed numerous small areas resembling minute abscesses with caseous contents, not unlike the picture seen in tuberculoma. Microscopically these areas appeared as foci of degenerated cells with groups of 10 to 20 schistosomal eggs in the center, and scattered eggs about the periphery. Some of these single ova were surrounded by a zone of granules with crenated margins. The abscess

291. Greenfield, J. G., and Pritchard, B.: Brain 60:361, 1937.

areas were surrounded by, first, a zone of fibroblasts and endothelial cells, with occasional giant cells, lymphocytes and plasma cells. This zone contained many small blood vessels and occasionally masses of endothelial cells. There was a gradual change to normal brain tissue, but for a considerable distance all the vessels were heavily infiltrated with mononuclear cells.

Other authors have believed that the lesions of the nervous system were due to embolic ova. Tsunoda and Shimamura<sup>292</sup> in 1906 described a case in which a disturbance of speaking and tremors of the arms and legs were noted for several months. Later the patient suffered from headaches, loss of memory, changes in reflexes, epileptiform seizures and right hemiplegia. On examination the authors found numerous sclerotic nodules in the cortex and the white matter and a walnut-sized area of softening in the region of the internal capsule. Ova of *S. japonicum* were noted in these areas embedded in dense neuroglia or surrounded by foreign body tubercles with necrosis and destruction of tissue. There was a general increase of neuroglia, especially along the small vessels, and fiber degeneration in the right pyramidal tract was noted. Eggs were also found in the choroid plexus and the spinal cord. Ferguson<sup>293</sup> reported finding the eggs of *S. haematobium* in both the brain and the spinal cord. In 1 case, the clinical symptoms resembled those of multiple sclerosis and the postmortem examination showed eggs in the spinal cord. The usual pathologic picture was that of a completely calcified egg surrounded by well marked signs of neuroglial hypertrophy. Edgar<sup>294</sup> observed a case in which convulsions and symptoms similar to those in the cases of Greenfield and Pritchard<sup>291</sup> were present. At operation a sharply outlined yellowish tumor, an inch in diameter, consisting of ova of *S. japonicum*, was found under the parietal bone. Vitug, Cruz and Bautista<sup>295</sup> described a case with sudden onset of convulsions in which eggs of *S. japonicum* were found in various tissues, including the brain. Here they were found in granular areas in the pia-arachnoid, the cortex and the white matter and in the capillaries of the choroid plexus. The brain showed formation of pseudotubercles with multi-nucleated giant cells about nests of eggs. There were apparent diffuse increase of glial tissue and fibrosis.

Involvement of the spinal cord by schistosome ova with production of transverse myelitis was first observed by Ferguson.<sup>293b</sup> Müller and

292. Tsunoda, T., and Shimamura, S.: Wien. med. Wchnschr. **56**:1681, 1906.

293. Ferguson, A. R.: (a) Glasgow M. J. **79**:4, 1913; (b) J. Roy. Army M. Corps **29**:57, 1917.

294. Edgar, W. H.: J. Roy. Nav. M. Serv. **22**:150, 1936.

295. Vitug, W.; Cruz, J. R., and Bautista, L. D.: J. Philippine Islands M. A. **21**:291, 1941.

Stender<sup>296</sup> described the pathologic changes in a case of schistosomiasis with transverse myelitis beginning in the upper thoracic part of the cord. They noted numerous pseudotubercles scattered throughout the cord, most frequently in the anterior horns and in the lumbar and lower thoracic segments of the cord. A typical pseudotubercle contained an egg of *S. mansoni* at the center, but many did not. Near every egg was a giant cell, and in some cases giant cells completely surrounded the egg. A zone of necrotic tissue surrounded the ovum, and outside of this was a border of lymphocytes and plasma cells. Destruction of tissue, with production of glial cells and of macrophages and fat granule cells, surrounded the pseudotubercles. The ganglion cells suffered little change. The spinal fluid showed an increase of cells and a marked increase of protein. The authors surmised that the necrotic zone about the egg was caused by a toxin liberated by the egg. They also surmised that the eggs reached the cord through venous anastomoses between the portal veins and the vertebral plexus of veins. Day and Kenawy<sup>297</sup> recorded a case of myelitis caused by *S. haematobium*. Ova were found in the lumbar enlargement in the anterior horns and the lateral columns, surrounded by granulation tissue made up of histiocytes and lymphocytes. The nerve cells near the ova showed varying degrees of degeneration, possibly due to diffusion of toxins from the eggs. Similar cases were reported by Bayoumi<sup>298</sup> and Espin.<sup>299</sup>

*Paragonimiasis*.—Paragonimiasis is caused by an infection with the fluke *Paragonimus westermani*, which inhabits the tissues of the lungs of man and various mammals. It is present throughout the Orient and has also been recorded from parts of Africa and South America. In some districts of Japan 40 to 50 per cent of the population is infected. Two intermediate hosts are required in the life cycle, snails and then crabs and crayfish. Human infection is acquired through eating crayfish containing encysted larvae.

Not infrequently the larvae migrate and develop in other tissues, including the brain. Cysts similar to those found in the lungs develop in the intracranial cavity, and symptoms of an intracranial neoplasm result. The first of a number of such cases was described by Otani in 1887. His patient had lung fluke infection complicated by epileptiform seizures, apathy and confusion. Autopsy revealed a mass as large as a hen's egg in the right frontal lobe and a smaller area grown to the dura in the occipital lobe. Cut sections revealed a mass of communicating cysts

296. Müller, H. R., and Stender, A.: Arch. f. Schiffs- u. Tropen-Hyg. **34**:527, 1930.

297. Day, H. B., and Kenawy, M. R.: Tr. Roy. Soc. Trop. Med. & Hyg. **30**:223, 1936.

298. Bayoumi, M. L.: J. Egyptian M. A. **22**:457, 1939.

299. Espin, J.: Rev. Policlín. Caracas **10**:245, 1941.

containing dark brown fluid. A fluke was seen in the fluid and another in normal brain tissue. Yamigawa<sup>300</sup> reported a similar case with convulsions and hemiparesis of two years' duration. Pathologic examination revealed groups of fluke eggs in the cortex surrounded by nodules consisting of an atrophic center and a surrounding round cell infiltration. The surrounding vessels were frequently increased and showed proliferation of their walls. Giant cells were also present. Taniguchi<sup>301</sup> described the case of a 17 year old girl with symptoms of epilepsy, chorea and athetosis, weakness of the left side and intellectual deterioration. Cut sections revealed in the right frontoparietal and temporal lobes several groups of cysts filled with thick brownish liquid containing fluke eggs. The microscopic examination revealed nodules and cyst walls of two layers—an inner, somewhat fibrous layer and an outer one of dense round cell infiltration. A diffuse glial increase was noted in the surrounding normal brain tissue, and the involved pyramidal tract showed degeneration. Musgrave<sup>302</sup> reported on a case of epilepsy in which the dura was thickened and contained parasitic abscesses similar to those found in the lung. A few eggs were seen in the choroid plexus also.

In 1921 Kawamura and Yamaguchi<sup>303</sup> reviewed 38 cases of cerebral paragonimiasis in the literature and added 37 of their own. With regard to children, they stressed the occurrence of epileptiform seizures, transient paralyses and other neurologic disturbances. In 1 case which they examined pathologically the patient had a nine year history of convulsions and deterioration. Several nodules were seen on the surface of the brain, and cut sections revealed numerous cystlike nodules and areas of softening in the cortical layers. The cysts were encapsulated by a fibrous membrane, and the contents contained lime granules, cholesterol crystals, eggs, giant cells and amorphous material. Round cell proliferation and increased glial cells surrounded the capsule. No flukes were found. Kimura<sup>304</sup> reported on a case in which mental peculiarities and hallucinations were noted before death. Autopsy revealed many cysts throughout the brain with atrophy of the occipital lobe.

Yokogawa and Suyemori<sup>305</sup> studied the infection experimentally and concluded that the main pathologic changes were probably due to flukes migrating in and out of the brain by way of the loose connective tissue in the neck.

300. Yamigawa, K.: *Virchows Arch. f. path. Anat.* **119**:447, 1890.

301. Taniguchi: *Arch. f. Psychiat.* **38**:100, 1904.

302. Musgrave, W. E.: *Philippine J. Sc.* **2**:15, 1907.

303. Kawamura, R., and Yamaguchi, M.: *Japan M. World* **1**:1, 1921.

304. Kimura, O.: *Mitt. a. d. Path. Inst. d. k. Univ. zu Sendai* **1**:1, 1919.

305. Yokogawa, S., and Suyemori, S.: *Am. J. Hyg.* **1**:63, 1921.

## Book Reviews

**Physical Chemistry of Cells and Tissues.** By Rudolf Höber, M.D., department of physiology, University of Pennsylvania, Philadelphia. With the collaboration of: David I. Hitchcock, Yale University Medical School; J. B. Bateman, Mayo Clinic, Rochester, Minn.; David R. Goddard, University of Rochester, and Wallace O. Fenn, University of Rochester. Price \$9. Pp. 676 with 70 illustrations. Philadelphia: The Blakiston Company, 1945.

This is a difficult book to characterize. It succeeds "*Physikalische Chemie der Zelle und der Gewebe*" by the distinguished senior author of the present volume, now of the University of Pennsylvania, but is only distantly related to it. The book does not purport to be a systematic review of physical chemistry as applied to the problems of cells and tissues, as its predecessor was, but is devoted rather to an attempt to bring certain problems of general physiology into closer relationship with physical-chemical science, the authors having deliberately chosen "a new start from a new base line." The volume suffers from the lack of integration common to books by multiple authors. This is in part attributable to incompleteness, since, according to the preface, it was impossible, owing to the war, to carry out the original plan in its entirety.

The first 216 pages of a total of 635 pages of text are devoted to a review of certain aspects of physical chemistry. Section 1, by David I. Hitchcock, is concerned with "Selected Principles of Physical Chemistry." Section 2, by J. R. Bateman, treats of "Large Molecules; Their Physicochemical Properties and Their Architectural and Functional Significance in Living Matter." Of the remaining six sections, four are contributed by Dr. Höber. Three of these, including one which is a short introduction, are concerned primarily with permeability: section 4, with the permeability of cells; section 8, with studies of permeability as applied to the functions of tissues, such as intestinal absorption, the formation of urine and the elaboration of gastric juice. Section 5 deals with the effects on cells of experimental changes in their environment, and is also largely concerned with permeability. Interpolated between section 5 and section 8, the reason for this arrangement not being clear to the reviewer, are section 6, by David R. Goddard, entitled "The Respiration of Cells and Tissues," and section 7, by Wallace O. Fenn, entitled "Contractility."

As stated in the introduction to section 1, "the treatment of physicochemical principles is based on the assumption that its readers already have some knowledge of the subject." Inspection of the first few pages of the text will reveal that this implies a considerable working knowledge of the calculus, differential and integral. The book is definitely not for beginners. Not only is the approach to physical chemistry somewhat advanced, but the reader is largely left to perceive for himself the applicability of the facts presented. Also the "selected principles" omit certain subjects, such as surface phenomena, which could have been reviewed with profit.

The remainder of the book falls into a series of three monographs, those listed above by Drs. Goddard and Fenn and a longer one, chiefly on the subject of permeability, by Dr. Höber. As to the section by Dr. Fenn, it may be said quite simply that it is of the excellent quality one has learned to expect from this author. It will be of interest to every student of general physiology and is integrated cellular physiology at its best. Being largely qualitative in its treatment, it may be read as an introduction to its subject matter and to the book itself. Dr. Goddard deals chiefly with intermediate carbohydrate metabolism. He has invoked physical-chemical principles whenever this was necessary, and in this way his section gives meaning to some parts of section 1. His presentation is excellent in every way and fulfills the promise of the title of the book. The reader would perhaps gain

the utmost from the book by reading sections 6 and 7 first, referring back to sections 1 and 2 when he feels the need of consulting a more detailed presentation of physical-chemical principles.

Dr. Höber's sections, viewed as a monograph, suffer from a lack of orientation to a unified concept of permeability. In fact, as the author states, the term "permeability" is used in section 8 in a sense (actually two senses) different from that (actually those) used in the earlier sections, and it is not always clear which sense is intended. The material on the subject of permeability is here for the student of the subject, although not, without considerable effort, for the uninitiated. The final chapter of the book, which is part of Dr. Höber's contribution, is a timely treatment of the energetics of active transfer, the transferring devices and their mechanics.

As Dr. Höber points out in the preface, "during the last four decades a tremendous revolution in our conception of the inorganic world has taken place, which during the last twenty years has progressively seized the aspect of the world of organisms." This book represents a noteworthy attempt to reorient certain fields of general physiology to the newer physics and physical chemistry, and as such will be of interest to biologists generally.

**Pulmonary Edema and Inflammation: An Analysis of Processes Involved in the Formation and Removal of Pulmonary Transudates and Exudates.** By Cecil K. Drinker, M.D., D.Sc., professor of physiology, Harvard University School of Public Health, Boston. Cloth. Pp. 106, with 27 figures and illustrations. Price, \$2.50. Cambridge, Mass.: Harvard University Press, 1945.

This monograph is composed of the Nathalie Gray Bernard Lectures delivered at the Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C., in December 1944, together with a fifth chapter on artificial respiration. It contains experimental results obtained by the author and his students, some of which have not previously been published. The chapters take up in succession the relation of lung structure to edema and inflammation, physiologic factors in pulmonary edema and inflammation, breathing movements and pulmonary edema, preventive and therapeutic measures in asphyxiating pulmonary disease, and artificial respiration.

The book is concerned more with pulmonary edema than with inflammation, and in inflammation the fluid component of the exudate is stressed to the exclusion of the cellular phenomena. Increased permeability of capillary endothelium, caused by anoxia, is considered more important in the development of pulmonary edema than are changes in pulmonary capillary pressure. A thiourea derivative which selectively affects the endothelium of the capillaries of the lung was also used to produce pulmonary edema. It is hoped that the withholding of the name of this important compound is explained by the exigencies of war. This book is highly recommended to all who are concerned with normal and abnormal states in the lung.

## Notes and News

**Appointments.**—Francis C. Tucker has been appointed assistant pathologist at St. Luke's Hospital, Chicago.

Lawrence W. Smith has resigned as professor of pathology in the Temple University School of Medicine, Philadelphia.

Philip R. White, since 1938 associate in the department of animal and plant pathology of the Rockefeller Institute of Princeton, N. J., has become a member of the institute for cancer research at Lankenau Hospital, Philadelphia.

Arthur C. Allen, who for the past three and a half years has served as pathologist at the Army Institute of Pathology, Washington, D. C., has recently been appointed associate professor of pathology at New York Medical College.

Crichton McNeal, stationed at the Army Institute of Pathology, Washington, D. C., has been appointed instructor in pathology at the University of Utah.

Dr. V. D. Sneeden has been released from military service and has returned to the department of pathology of the University of Oregon Medical School as associate professor.

Ida A. Bengston and Alice C. Evans, bacteriologists of the United States Public Health Service, have retired after many years of work in the National Institute of Health.

**Deaths.**—Newton G. Evans, professor of pathology in the College of Medical Evangelists since 1914 and for many years pathologist of the Los Angeles County Hospital, died Dec. 19, 1945, at the age of 71.

Henry B. Ward, professor emeritus of zoology in the University of Illinois, well known for his work in parasitology, died Nov. 30, 1945, at the age of 80 years.

**Society News.**—The Association of Pathologists of West Virginia has been organized with C. C. Fenton, Morgantown, as president and W. T. McClure, Wheeling, as secretary-treasurer. The first meeting will be held at Huntington, W. Va., May 14, 1946, in conjunction with the State Medical Association.

The American College of Physicians will hold its 1946 meeting in Philadelphia, May 13 to 17 inclusive, at the Municipal Auditorium.

The American Association of Pathologists and Bacteriologists will meet at the University of Chicago, Friday and Saturday, March 8 and 9, 1946.

The Federation of American Societies for Experimental Biology will meet in Atlantic City, N. J., beginning on Monday, March 11, 1946.

The thirty-seventh annual meeting of the American Association for Cancer Research will be held in Atlantic City, N. J., Monday and Tuesday, March 11 and 12, 1946, concurrently with the opening meetings of the Federation of American Societies for Experimental Biology. The Ambassador Hotel will be headquarters.

**Awards.**—Wendell M. Stanley, of the Rockefeller Institute of Princeton, N. J., has been awarded the 1946 William H. Nichols Medal of the New York Section of the American Chemical Society in recognition of his work on the chemistry of viruses.

The Copley Medal of the Royal Society has been awarded to O. T. Avery, of the Rockefeller Institute of New York, for his introduction of chemical methods in the study of anti-infectious immunity.

**Postgraduate Refresher Course for Pathologists Returning from Military Service.**—The American Society of Clinical Pathologists announces a postgraduate refresher course for pathologists returning from military service, to be held in Chicago, at the Drake Hotel, March 2 to 5, 1946.

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## DISTRIBUTION OF LIPASE IN THE TISSUES UNDER NORMAL AND UNDER PATHOLOGIC CONDITIONS

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CHICAGO

THIS paper gives a description of pictures obtained in various organs and tissues with my technic for the microtechnical visualization of sites of lipase activity.<sup>1</sup> The theoretic background of the method was given in the original paper.

### MATERIAL AND TECHNIC

A number of normal tissues and organs of the following species were examined: man, rhesus monkey, dog, rabbit, guinea pig and rat. The pathologic material consisted partly of surgical specimens, partly of autopsy material.

The original technic was used in this work except for minor modifications and the introduction of a new substrate in addition to the previous ones. It was found that tissue blocks infiltrated with acetylcellulose before paraffin embedding offer definite advantages. This substance largely eliminates shrinkage; it keeps the sections, which otherwise have a tendency to break up into pieces when floated on lukewarm water, firmly together; it also obviates the necessity of protecting them with a layer of collodion before staining. Nitrocellulose is unsuitable for this purpose because, owing to its solubility in a mixture of acetone and benzene, most of it will be removed from the tissue block when it is transferred from acetone to benzene. The maleate buffer recommended in the original paper can be dispensed with altogether, and, instead, the incubating mixture can be neutralized with tenth-normal sodium hydroxide to  $p_{\text{H}}$  6.8 to 7.4, bromthymol blue being used as an indicator. The new substrate is "Product 81", a stearic ester of comparatively short-chained polyglycols, manufactured by the Onyx Oil and Chemical Co., Jersey City, N. J.

The modified technic is as follows:

1. Fix thin slices of tissue in chilled acetone for twelve to twenty-four hours in the ice box. Fresh tissues are preferable, although entirely satisfactory results can be obtained with tissues kept unfixed at ice box temperature as long as twenty-four hours.
2. Dehydrate in two changes of absolute acetone, twelve to twenty-four hours each, at room temperature.

From the Department of Medicine, University of Chicago.

This work has been done under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

1. Gomori, G.: Proc. Soc. Exper. Biol. & Med. 58:362, 1945.

3. Impregnate in a 5 per cent solution of acetylcellulose in acetone for twenty-four hours. Eastman's cellulose acetate "high acetyl, low viscosity, no. 4644" was found satisfactory.
4. Drain pieces and transfer them into two changes of benzene, one hour each.
5. Embed in paraffin at a temperature of 56 to 62 C., using two changes, one to one and one-half hours each.
6. Cut sections at 4 to 8 microns, float them on lukewarm ( $\pm$  35 C.) water and mount on slides.
7. Run slides through xylene and graded alcohols to distilled water.
8. Incubate at 37 C. for six to twelve hours in the following mixture:

Stock solution I:

Glycerin	150 cc,
10 per cent calcium chloride	
Half-molar maleate buffer $p_H$ 7 to 7.4	50 cc. each,
Distilled water to make	1,000 cc.

Stock solution II:

Five per cent aqueous solution of Tween 40 or Tween 60 (obtainable from Atlas Powder Co., Wilmington, Del.) or of Product 81.

With merthiolate added in a concentration of about 0.02 per cent, both stock solutions keep in the ice box for months.

Before use, add 2 cc. of stock solution II to 50 cc. of stock solution I. The maleate buffer is prepared by dissolving 5.8 Gm. of maleic acid in a mixture of 94 cc. of normal (4 per cent) sodium hydroxide and 6 cc. of distilled water. As mentioned, it can be left out entirely, in which case the mixed incubating solution must be neutralized to the  $p_H$  specified.

9. Rinse slides in distilled water.
10. Transfer slides to a 1 to 2 per cent solution of lead nitrate for ten to fifteen minutes.
11. Rinse slides thoroughly in repeated changes of distilled water.
12. Transfer slides for one minute to a dilute solution of light yellow ammonium sulfide (a few drops to a Coplin jarful of distilled water). Sites of lipase activity will show up in a dark brown shade.
13. Wash under the tap. Counterstain with hematoxylin and, very lightly, with eosin.
14. Dehydrate in graded alcohols. Clear in gasoline or tetrachloroethylene (perchloroethylene). Mount in clarite dissolved in the same solvents. Do not use toluene or xylene, which will cause gradual fading of sections.

The method is reliable, not a single failure having been observed in working up over 100 cases. Its only shortcoming is an occasional failure to obtain good nuclear staining with hematoxylin, especially after the use of the Tweens, much more rarely with Product no. 81. The reason for this phenomenon is not understood; it may happen that of two consecutive serial sections treated in an identical way, one will show excellent, and the other very poor, nuclear staining.

Confusion may arise from the presence of dark brown pigments, such as melanin, lipochrome or hemosiderin, or of granular deposits of calcium salts, which in the finished sections may show up in a shade indistinguishable from that given by lipase. However, differential diagnosis is easy. Pigments are visible in unincubated sections, whereas lipase is not. The prussian blue reaction in a duplicate slide will eliminate the error due to hemosiderin, and the von Kossa silver stain, that due to the presence

of calcium salts. The latter can also be removed by treating the slide for ten to fifteen minutes with a citrate buffer of  $p_{\text{H}}$  4.5 to 5.

#### RESULTS IN GENERAL

The activity of lipase, unlike that of the phosphatases, which give diffuse staining, is morphologically associated with cytoplasmic granules, which in some tissues may be very coarse (fig. 1). The nuclei are unstained. The pattern of distribution obtained in the same organ in different individuals of the same species is remarkably constant, although the intensity of the reaction may vary considerably. In different species the same organ may show different patterns. All substrates used gave identical pictures.

#### OBSERVATIONS ON NORMAL TISSUES AND ORGANS

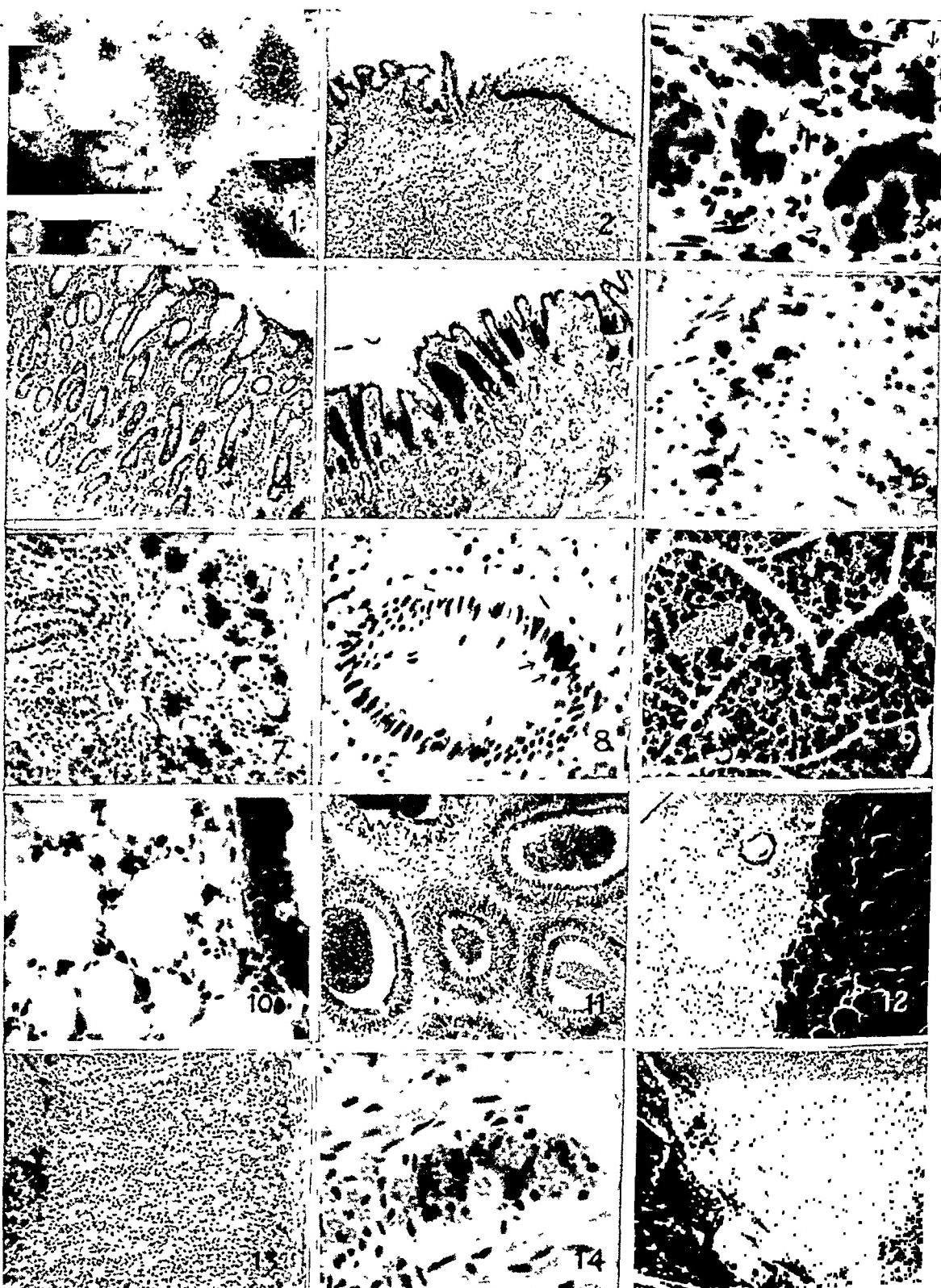
*Vascular and Hemopoietic System.*—In all species the blood vessels, as a rule, show no lipase activity. Occasionally, however, a positive reaction is obtained in the intima and the adventitia of medium-sized and large arteries, especially in pregnant rats. Blood cells of all kinds show no reaction. This is at variance with the findings of Barnes,<sup>2</sup> who was able to demonstrate minimal amounts of lipase in lymphocytes and leukocytes. The amount of enzyme present in these cells is apparently below the threshold of sensitivity of the histochemical method. Spleen, lymph nodes and bone marrow are also without evidence of lipase activity.

*Digestive System.*—The salivary glands of the guinea pig and the rat were examined. In the guinea pig the cells of the serous glands are intensely lipase positive, those of the mucous glands much less so. In the rat there are scattered cells outside the alveoli themselves, both in serous and in mucous glands, which contain positively staining granules. These cells are tentatively identified as basket cells. In both species the excretory ducts are entirely without evidence of this activity.

The deep layers of the epithelium of the esophagus contain lipase in all species examined. In animals the positive reaction is quite uniform, while in man it is patchy, being present in some regions and absent in others. An intense positive reaction is obtained in the esophagus in pig embryos of a crown-breath length of 11 cm. and above (fig. 2).

The distribution of lipase in the stomach varies greatly with different species. In the human stomach the fundic portion shows lipase in the zymogenic cells only, especially in the depths of the glands (fig. 3). The parietal cells are entirely without evidence of it. The antral and pyloric regions, together with the duodenum, show intensely stained cells, irregularly scattered in the depths of the glands (fig. 4).

2. Barnes, J. M.: Brit. J. Exper. Path. 21:264 1940.



(See legends on opposite page)

With routine stains these lipase-containing cells are indistinguishable from the majority of those which show no reaction. In the dog the epithelium of the surface and of the necks of the glands shows lipase activity (fig. 5), much more in the fundic than in the antral portion. There is no reaction in the depths of the crypts. In addition, there are minute groups of small, obviously not epithelial cells present in the stratum proprium of the stomach, the duodenum and the small intestine showing an intense reaction (fig. 6). The nature of these cells is not clear. The duodenal and jejunal villi are without evidence of lipase activity. In the rabbit, the guinea pig and the rat the entire stomach appears free of lipase, but the epithelium of the duodenal and jejunal villi shows a positive reaction, with the exception of the goblet cells. In the rabbit, around the periphery of Brunner's glands (fig. 7) there are crescent-shaped groups of cells showing intense staining.

The colon and the appendix of man present a picture similar to that of the antrum of the stomach except for the fact that the lipase-containing cells are much more sparse (fig. 8). In all other species, the rhesus monkey excepted, the colon shows no lipase activity. In the rhesus monkey the entire epithelium of the colon, goblet cells included, is lipase active.

The liver presents an intensely positive reaction in all species examined. Whereas in most species the reaction is of a fairly uniform intensity throughout the lobules, in the rat the central areas are often much more intensely stained than the peripheral ones. The connective

#### EXPLANATION OF PLATE.

Fig. 1.—Coarse lipase-positive granules in a rabbit's liver.  $\times 266.5$ .

Fig. 2.—Cardia of an 11 cm. (crown-breech length) pig embryo. Deep layers of esophageal lining show intense lipase activity.  $\times 66.5$ .

Fig. 3.—Fundic portion of a human stomach, showing a positive reaction in zymogenic cells and absence of reaction in parietal cells (arrows).  $\times 266.5$ .

Fig. 4.—Antral portion of a human stomach. Note the scattered dark cells in the depths of the crypts.  $\times 66.5$ .

Fig. 5.—Fundic portion of a dog's stomach showing a surface reaction.  $\times 66.5$ .

Fig. 6.—Fundic portion of a dog's stomach. Scattered dark cells are seen in the stratum proprium.  $\times 185$ .

Fig. 7.—Distal portion of a rabbit's duodenum. Crescent-shaped groups of cells show lipase activity.  $\times 133$ .

Fig. 8.—Human appendix. Two cells showing lipase activity are shown by arrows.  $\times 266.5$ .

Fig. 9.—Pancreas of a rat.  $\times 66.5$ .

Fig. 10.—Lung of a rat. An intensely positive reaction is present in the bronchial epithelium and in the septal cells.  $\times 266.5$ .

Fig. 11.—Epididymis of a dog.  $\times 66.5$ .

Fig. 12.—Ovary of a dog, showing part of a corpus luteum.  $\times 66.5$ .

Fig. 13.—Human adrenal gland containing in the zona reticularis a number of cells showing the specific staining.  $\times 66.5$ .

Fig. 14.—A ganglion of Auerbach's plexus in a rhesus monkey. The ganglion cells show no lipase; the satellite cells present a positive result.  $\times 266.5$ .

Fig. 15.—Adrenal gland of a rat with the periadrenal fat intensely stained.  $\times 66.5$ .

tissue of the liver is entirely without evidence of lipase activity, and so are the Kupffer cells. In the rat the lining of the bile ducts reveals a positive reaction, but in all other species it shows little or no lipase activity.

In all species the pancreas shows intense staining of the zymogen granules (fig. 9) while the peripheral portion of the acinous cells may be unstained. The secretions within the ducts are also intensely reactive. The islets of Langerhans are nonreactive throughout. The addition of bile salts to the incubating mixture causes a considerable intensification of the lipase picture of the pancreas, while it reduces the intensity of the reaction in all other organs. This is in complete agreement with the findings of Willstätter and Memmen.<sup>3</sup>

*Respiratory System.*—The bronchial epithelium shows a positive reaction in all species examined, although in man in a patchy way. Some of the bronchial glands also are stained. The septal cells are intensely stained in a uniform and selective way in the rat (fig. 10) and in the guinea pig, less intensely in the rabbit and inconstantly in the dog. In man, the attached septal cells are without lipase activity but the desquamated ones show a positive reaction.

*Uropoietic System.*—In man the kidney is entirely without lipase activity. In the dog the cells of the convoluted tubules stain very weakly, but the straight portion of the proximal tubules down to the transition into Henle's loop shows more or less intense activity of lipase. The tubules distal to this transition point do not show lipase activity. The epithelium of the renal pelvis and of the urinary bladder presents a positive result. In the guinea pig the entire kidney, as a rule, is free from lipase activity, although occasionally the convoluted tubules may be slightly stained. In the rabbit and the rat, both the proximal and the distal convoluted tubules show lipase activity, while Henle's loops and the collective tubules are without evidence of it. This is in agreement with the data of Weil and Jennings.<sup>4</sup> The epithelium of the renal pelvis and of the urinary bladder shows a positive reaction in the guinea pig and the rabbit and no reaction in the rat.

*Male Genital System.*—In man, the seminiferous tubules show no lipase activity, while the interstitial cells show a positive reaction. In the rabbit and the rat the picture is very similar. In the dog the Sertoli cells contain coarse lipase-positive granules; the interstitial cells contain none. The testis of the guinea pig is entirely without evidence of lipase activity. The epithelium of the epididymis shows a positive result to a varying extent in all species examined; the brush border may show especially heavy staining (fig. 11). In the guinea pig,

3. Willstätter, R., and Memmen, F.: Ztschr. f. physiol. Chem. **138**:216, 1924.

4. Weil, L., and Jennings, R. K.: J. Biol. Chem. **139**:421, 1941.

the epithelium of only the distal portion of the epididymis shows staining, while that of the proximal portion is entirely free from evidence of lipase activity. There is an interesting reciprocal relationship between lipase and alkaline phosphatase pictures in that the basement membrane of the lipase-containing distal part shows no phosphatase reaction, whereas that of the lipase-free proximal portion shows intense phosphatase activity.

The prostate in man reveals no evidence of lipase activity. In the dog the lining of the excretory ducts and of some of the glands shows moderate staining.

*Female Genital System.*—In woman, the ovary and the nonpregnant uterus are without lipase; the decidua of the pregnant uterus shows a patchy lipase reaction. In the dog, the rabbit, the guinea pig and the rat, the cells of the corpora lutea contain very coarse lipase-positive granules. Especially intense is the reaction in the dog's ovary (fig. 12). In addition, an intense reaction was found in the theca of ripening follicles in 1 pregnant rat. The uterus and the tubes are without evidence of lipase activity in all animal species.

*Endocrine Glands.*—The adrenal gland shows a marked species difference in the lipase picture. In man a varying number of cells in the zona reticularis are lipase active (fig. 13). In the dog the entire zona glomerulosa and irregular groups of cells in the zona reticularis show the reaction. The guinea pig's adrenal gland appears entirely free of lipase. In the rabbit there is an inconstant patchy reaction of moderate intensity extending from the zona glomerulosa into the zona fasciculata. The rat's adrenal gland is either entirely without lipase activity or shows a very faint reaction in the zona glomerulosa and the outer portion of the fasciculata. The medulla in all species reveals no lipase.

The thyroid gland, the parathyroid gland and the thymus show no lipase in any species. The pituitary gland was not examined.

*Nervous System.*—Tissues of the nervous system were not examined. The only positive finding was a distinct reaction in the satellite cells of the Auerbach plexus in the rhesus monkey (fig. 14) and a much less conspicuous but similar reaction in the rabbit.

*Muscles, Skin and Its Appendages.*—These organs are without evidence of lipase activity in all species.

*Connective Tissue.*—Groups of brown-staining fibroblasts are occasionally seen in various organs of all species. Especially in embryos, strands of intensely stained fibroblasts are seen in the walls of the gastrointestinal tract. The adipose tissue shows a consistently positive result in the rabbit and in the rat (fig. 15) and a negative result in the other species, although groups of moderately stained fat cells are sometimes observed in human adipose tissue.

## OBSERVATIONS ON PATHOLOGIC TISSUES AND ORGANS

*Miscellaneous Non-Neoplastic Changes (Inflammatory and Degenerative Processes; Granuloma).*—Inflammatory processes do not change the lipase picture unless they lead to necrosis. Necrotic tissue, regardless of its origin, contains no or very little lipase. No lipase is found in fatty degeneration of the kidney or of the myocardium. Fatty livers contain less lipase than normal ones. On the other hand, in atherosomatous patches of the aorta many of the fat-laden macrophages contain the brown-staining granules (fig. 16). The foam cells in 2 cases of Gaucher's disease were entirely without lipase activity.

In cirrhosis of the liver, the regenerated, large liver cells are more intensely stained than the rest of the lobule.

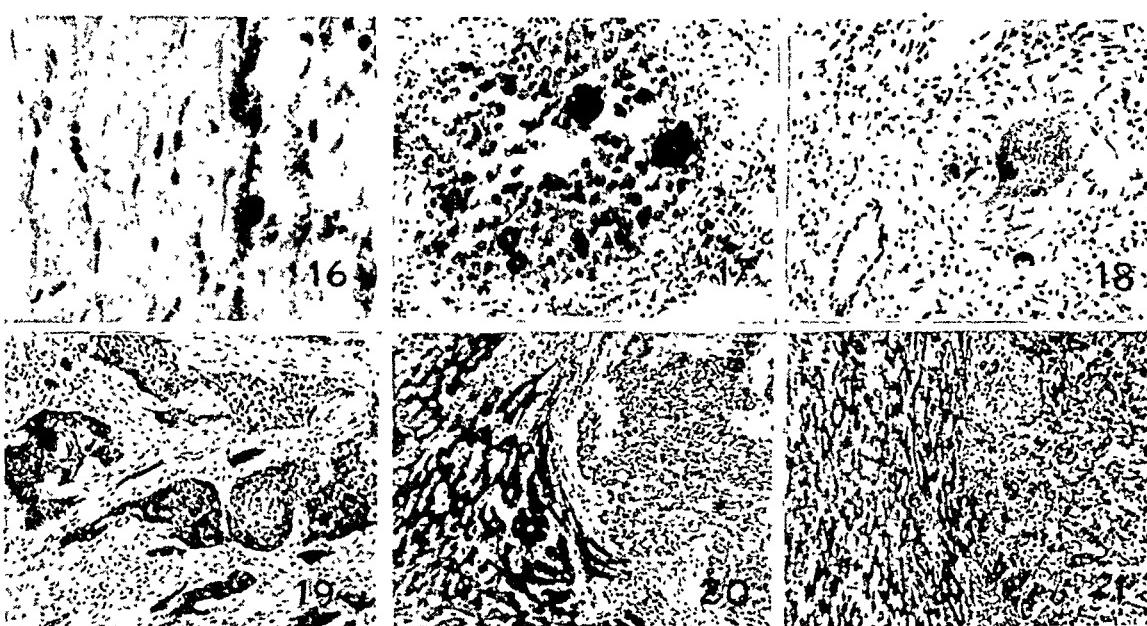


Fig. 16.—Atherosomatous patch of a human aorta with lipase active fat-laden macrophages.  $\times 266.5$ .

Fig. 17.—Tubercle in a rabbit's lung. The epithelioid and the giant cells show a positive result.  $\times 133$ .

Fig. 18.—Tubercle in a human kidney. A giant cell full of coarse lipase-active granules is seen.  $\times 133$ .

Fig. 19.—Squamous carcinoma of the esophagus. Most of the tumor cell nests show a positive reaction.  $\times 66.5$ .

Fig. 20.—Lipase-free hepatic metastasis of gastric carcinoma. The liver tissue itself shows positive staining.  $\times 66.5$ .

Fig. 21.—Hepatoma. Both the tumor and the liver tissue show a positive reaction.  $\times 66.5$ .

In tuberculosis, both the epithelioid and the giant cells are rich in lipase-positive granules, especially around the periphery of the tubercle. The reaction is intense and widespread in the rabbit (fig. 17), less intense and patchy in distribution in man (fig. 18). The necrotic

centers of tubercles contain no lipase. A picture similar to that seen in tuberculosis but of a lesser intensity is observed in sarcoidosis.

*Tumors.*—A large number of specimens of carcinoma and sarcoma of various organs were examined. All of them were found to be without lipase activity (fig. 19) except for 1 specimen of squamous carcinoma of the esophagus (fig. 20), 1 of hepatoma (fig. 21) and 1 somewhat atypical specimen of seminoma. The first and second specimens stained fairly uniformly, while in the third the seminoma cells themselves were without evidence of lipase activity but the stroma contained a large number of scattered spindle-shaped and polyhedral cells, the nature of which could not be established, which showed presence of lipase.

## EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

### XII. Atheromatosis in Dogs Following Repeated Intravenous Injections of Solutions of Hydroxyethylcellulose

W. C. HUEPER, M.D.  
NEW YORK

IN a series of experimental studies<sup>1</sup> it was shown that the prolonged parenteral introduction of large amounts of colloidal solutions of macromolecular carbohydrates (polyvinyl alcohol, methyl cellulose, pectin, sodium cellulose glycollate, acacia) elicits in the arteries of dogs and rabbits atheromatous lesions which contain the injected foreign matter in foam cells. When, therefore, hydroxyethylcellulose (Cellosize, produced by the Carbide and Carbon Chemical Corporation) became available, it appeared to be of interest to test this substance for any potential atheromatogenic properties.

#### EXPERIMENTAL PROCEDURE

Hydroxyethylcellulose is a white powder which is manufactured in three grades of viscosity: (WS-20 having a viscosity of 11.3 centipoises at 20 C. as a 5 per cent aqueous solution, WS-100 having a viscosity of 68.7 centipoises under these conditions and WS-500 having a viscosity of 465 centipoises). The reactions of such solutions are slightly below the neutral point ( $p_{\text{H}}$  6.6 to 8.6). The viscosity of each of these solutions decreases with increasing temperature. The compound is more soluble in hot water than in cold. The solutions do not gel at higher temperatures as do solutions of methyl cellulose. The aqueous solutions are translucent and slightly turbid. When given orally to rats, the substance is less toxic than glycerin, according to the statement of the manufacturer. It does not exert any irritating effect when applied to the skin but has a mild irritating action on the conjunctiva of the rabbit.

For the purpose of the experiment, the three types of hydroxyethylcellulose were prepared in isotonic solution of sodium chloride. In these solutions the concentration of hydroxyethylcellulose was adjusted in such a way that their viscosity was five to six times that of normal plasma or ten to twelve times that of water. The solutions used had the following concentrations: High viscosity grade, 2.3 per cent; medium viscosity grade, 5.0 per cent, and low viscosity grade, 10.0 per cent. They were injected into the jugular veins of dogs in amounts of 50 cc. daily after being filtered, while hot, through filter paper in a Buechner filter. Two dogs were used for the testing of each of the three

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From the Warner Institute for Therapeutic Research.

1. (a) Hueper, W. C.: Arch. Path. **28**:510, 1939; (b) **31**:11, 1941; (c) **33**:1, 1942; (d) Am. J. Path. **20**:737, 1944; (e) Arch. Path. **34**:883, 1942; (f) Experimental Studies in Cardiovascular Pathology: XIV. Experimental Atheromatosis in Macacus Rhesus Monkeys, Am. J. Path., to be published; (g) ibid. **18**:895, 1942; (h) Arch. Path. **33**:267, 1942. (i) Hueper, W. C.; Landsberg, J. W., and Eskridge, L. C.: J. Pharmacol. & Exper. Therap. **70**:201, 1940.

types of solutions. The dogs ranged in weight from 7.5 to 12.9 Kg. and in age from 10 months to 3 years, estimated from the condition of their teeth at the start of the experiment. The animals were killed at the end of the experimental period by intravenous injection of a 10 per cent formaldehyde solution.

The dogs remained in good health during the experiment. There were no major fluctuations in weight. The injections were in general well tolerated. Vomiting was observed only a few times, occurring in each instance shortly after the injection. There were brief moderate rises in temperature on several occasions in spite of the fact that the solutions were prepared with pyrogen-free water.

Studies of blood were made directly before, and five, ten, thirty minutes, two, five and twenty-four hours after, the first injection and at weekly intervals thereafter when injections were made five times a week over periods of six to twelve weeks. They included erythrocyte and leukocyte counts, a differential count of leukocytes, determinations of hemoglobin, coagulation time, sedimentation rate, volume of packed blood cells and viscosity of plasma.

#### HEMATIC OBSERVATIONS

*Hydroxyethylcellulose WS-500* (high viscosity grade).—The 2 dogs which received injections of a 2.3 per cent solution of the highly viscous hydroxyethylcellulose for periods of six and seven weeks, respectively, exhibited the following acute hematic reactions during the twenty-four hour period after the first injection of 50 cc. of this material. The number of erythrocytes and the amount of hemoglobin underwent only minor and inconsistent fluctuations. The number of leukocytes dropped at the five minute test to about half of the original number and remained at this level for approximately two hours. The original level was again attained at the five hour test. There was a moderate decrease (33 per cent) in the number of the polymorphonuclear leukocytes during the leukopenic phase, which was associated with relative lymphocytosis. The fluctuations in coagulation time and volume of packed blood cells were insignificant. The erythrocytic sedimentation rate was markedly increased in the five minute test and remained high (peak 51 mm. with Wintrobe-Landsberg tube) in one animal up to the twenty-four hour test, while it returned to normal values in the twenty-four hour test in the other. The viscosity of the plasma increased in the five minute sample from an original value of 1.6 and 1.9, respectively, to 2.7 centipoises. At the five hour test the value was 2.35, and it remained at this level at the twenty-four hour test.

During the chronic phase of the experiment there were no appreciable changes in the number and the ratio of leukocytes and in the coagulation time. The number of erythrocytes, the amount of hemoglobin and the volume of packed blood cells dropped progressively until they reached at the end of the experimental period about one half of the original values. The sedimentation rate was consistently and considerably increased except at times when in one of the dogs the plasma viscosity reached values above 5 and the sedimentation rate decreased sharply. No such relation was found in the second dog. The viscosity of the plasma was definitely and increasingly elevated, reaching at times values of 6 and a peak value of 8.

*Hydroxyethylcellulose WS-100* (medium viscosity grade).—Two dogs received intravenous injections of 50 cc. of a 5 per cent solution of the medium viscous hydroxyethylcellulose five times weekly for a period of eleven weeks. The following acute hematic reactions were noted during the first twenty-four hours following the initial injection. At the ten minute test there was a drop in the

number of erythrocytes amounting to more than 2,000,000 cells in both dogs. This hemodilution remained in effect, though to a lessened degree, for over five hours, with almost original values prevailing at the twenty-four hour test. There was a corresponding reduction in the amount of hemoglobin during this period. The volume of packed red cells was paradoxically increased during the erythrocytic period and reached especially high values when the reduction of the number of red cells was most severe. A marked colloidoclastic leukopenic response appeared at the five minute test, at which the number of leukocytes was reduced to about one third of the original number. This reaction persisted for thirty to sixty minutes, after which the number of leukocytes returned to an approximately original level. There were no considerable shifts in the ratio of the various types of leukocytes during this leukopenic response except in 1 dog at the five minute test, when lymphocytosis existed. The coagulation time remained unchanged. The sedimentation rate was only moderately increased. The viscosity of the plasma rose from 2.1 and 2.2, respectively, to 2.75 and 3.0, respectively, at the five minute test, and remained between 2.7 and 2.3 for the next five hours, being in one of the dogs still elevated in the twenty-four hour test (2.5).

Repeated intravenous injections of solutions of the medium viscous hydroxyethylcellulose caused a moderate drop in the number of erythrocytes, the amount of hemoglobin and the volume of packed blood cells. It was found on several occasions that the volume of packed blood cells was abnormally high (up to 74 mm.) despite a definitely reduced number of erythrocytes (3,660,000). The sedimentation rate was moderately to considerably accelerated.

*Hydroxyethylcellulose WS-20* (low viscosity grade).—Two dogs received intravenous injections of a 10 per cent solution of the low viscous hydroxyethylcellulose for twelve weeks five times a week. One dog exhibited during the first twenty-four hours after the first injection a mild to moderate decline in the number of erythrocytes and in the amount of hemoglobin. There occurred, on the other hand, a marked increase in the volume of packed blood cells of this dog at the five and twenty-four hour tests, when this value stood at 71 and 73 mm., respectively, despite a slightly to moderately lowered number of red cells. No such reactions were found in the second dog. The number of leukocytes fell sharply in both dogs at the five minute test to one third and to one fourth, respectively, of the original value and remained reduced for ten minutes to two hours. This reaction was accompanied by a considerable increase in the number of lymphocytes and a corresponding drop in the number of neutrophilic leukocytes at several but not all tests showing a leukopenic response. The coagulation times of both dogs and the sedimentation rate of one dog remained unchanged. There was a moderate acceleration of the erythrocytic sedimentation in the second dog lasting for two hours. The plasma viscosity was mildly elevated directly after injection, but this fluctuation stayed within normal limits.

After repeated intravenous administrations of this material there developed toward the end of the experimental period a moderate degree of anemia. Again it was noticed that the values of the volume of packed blood cells were at times abnormally high in comparison with the lowered number of erythrocytes. But this discrepancy was not as marked and as frequent as in the previous series. The number of leukocytes and their ratio fluctuated within normal limits during the experimental period. There were no abnormal changes in the coagulation time. The sedimentation rate was mildly, but not always, increased. The viscosity of the plasma remained within the normal range in one dog but was elevated in the other dog, rising to a peak value of 3.25.

## ANATOMIC OBSERVATIONS

The postmortem examination of the 6 dogs did not reveal any considerable deviation from normal conditions with the exception of those of the spleens, which were mildly to moderately increased in size, pinkish red and medium firm in consistency. The livers were normal in size and appearance.

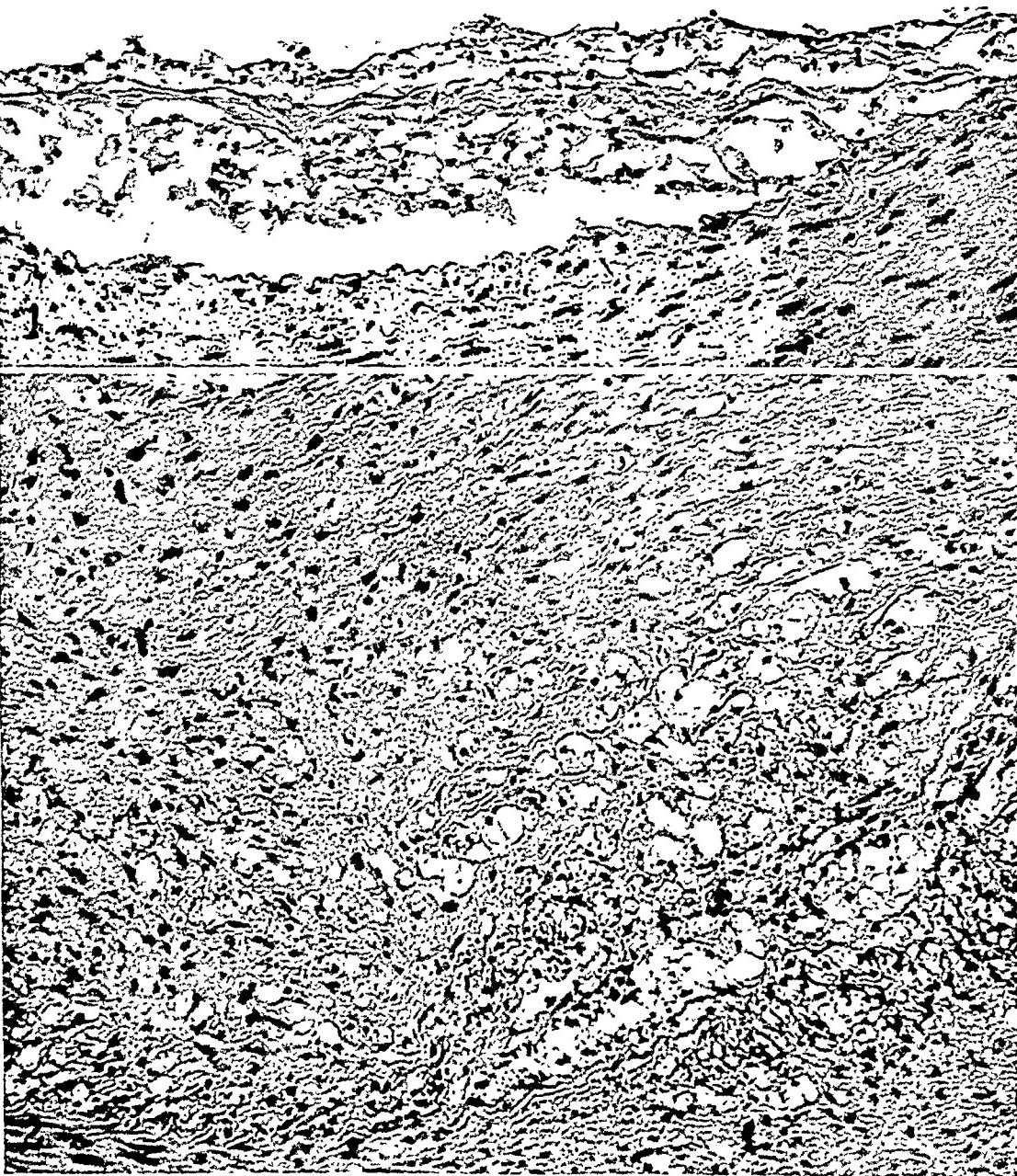


Fig. 1.—Thickened intima of an epicardial coronary artery, composed of foam cells and fibroblasts.

Fig. 2.—Large intimal cushion of the abdominal portion of the aorta, consisting mainly of fibrous tissue, with foam cells accumulated at its base.

A histologic examination was made of the following organs: thyroid gland, parathyroid glands, lungs, heart, aorta, carotid arteries, iliac arteries, brachial arteries, vena cava inferior, liver, spleen, pancreas, adrenal glands, kidneys, testis, prostate and bone marrow.

The organs of the 2 dogs which received the highly viscous hydroxyethyl-cellulose were essentially without any abnormal changes. The spleen of each showed a moderate increase of the reticular cells. The intima of the bulb of

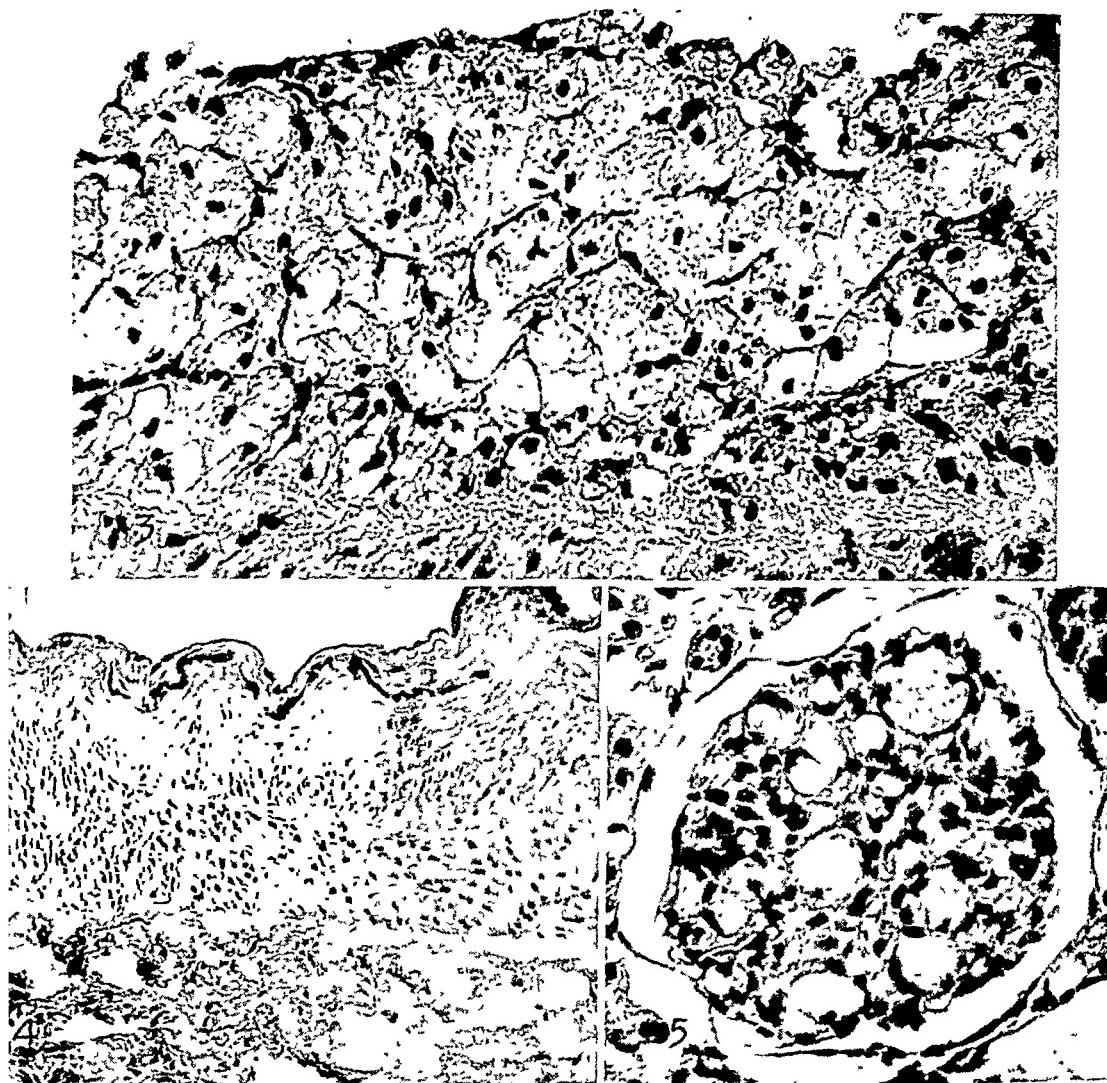


Fig. 3.—Foam cell proliferation of the endothelium of a medium-sized artery, forming a thick cushion.

Fig. 4.—Hyalinization and calcification of the media involving the elastica of a medium-sized artery.

Fig. 5.—Glomerulus with cystically dilated capillary loops containing homogeneous matter.

the aorta was locally thickened and consisted there of an edematous cushion of hydropic cells. There were no atheromatous lesions or other changes in the aorta and the arteries studied. The liver cells exhibited coarsely flaky cytoplasm.

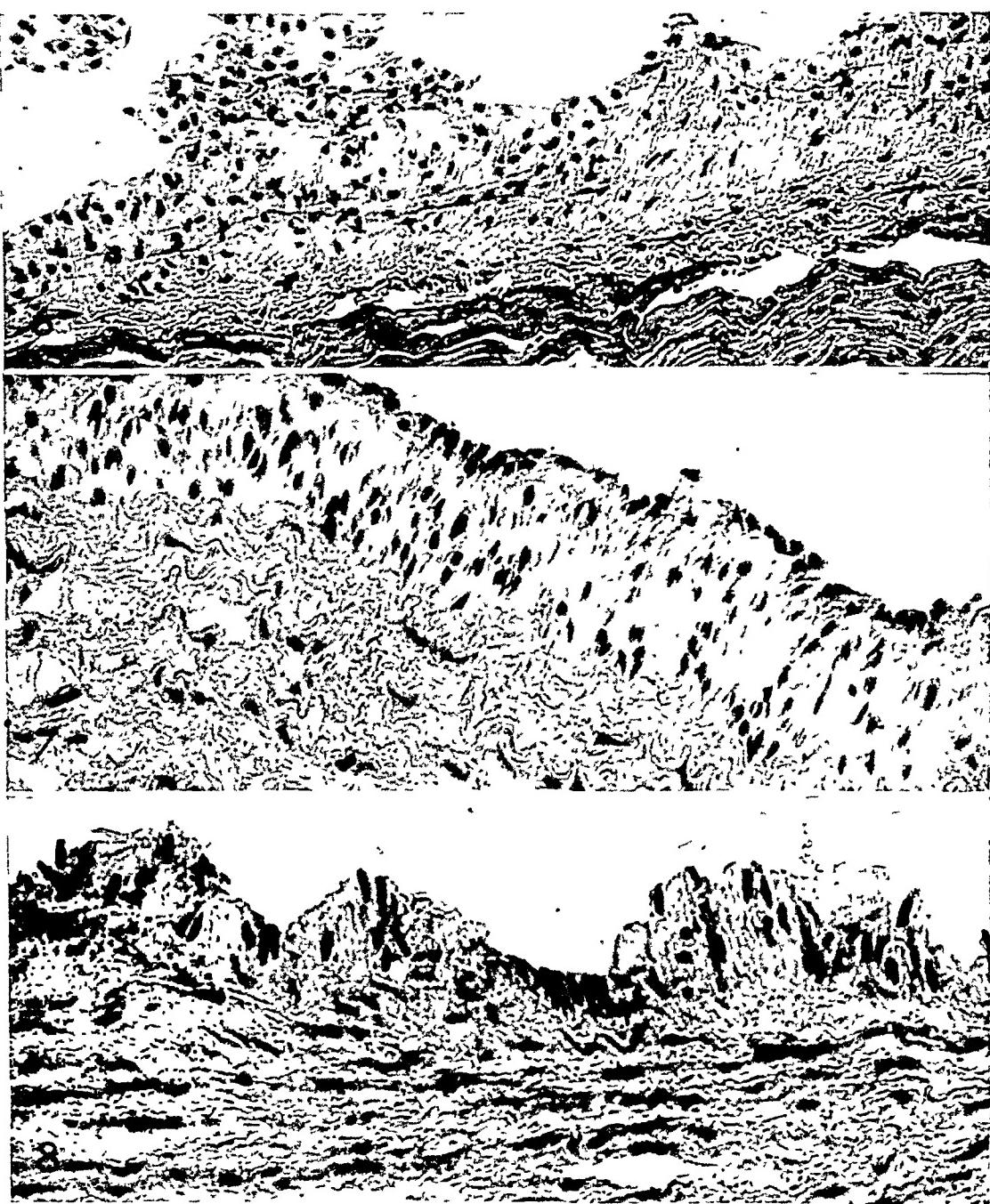


Fig. 6.—Foam cell proliferation of the endothelial lining of the left ventricle.

Fig. 7.—Crowding of endothelial cells and hyaline thickening of the intima, which contains mononuclear cells, of the ascending part of the aorta.

Fig. 8.—High cuboidal and cylindric endothelial cells forming small tonguelike cushions in the thoracic and abdominal portions of the aorta.

A few small interstitial round cell infiltrations were present in the kidneys of one dog and small calcium casts in the medullary tubular lumens of the kidneys of the other.

The parenchymatous organs of the 2 dogs which received injections of solutions of the medium viscous hydroxyethylcellulose were also in general without any appreciable abnormal reactions. A giant cell granuloma projecting like a broad-based papilloma into the lumen of a pulmonary vessel was noted in one dog. The cytoplasm of the hepatic cells was coarsely flaky. The spleen of each animal showed an increase of the reticular cells. In both dogs the tubular lumens in the renal medulla contained calcium casts. The renal cortex had a few interstitial round cell infiltrations in one dog. An appreciable accumulation of foam cells was found beneath the endothelium of a pericardial coronary artery of this dog (fig. 1). In both dogs the aortas revealed in the bulbar portion narrow cushions of hydropic tissue in which mononuclear cells were embedded. The upper end of the aortic bulb of one dog contained a large calcified cartilaginous focus in the media. The abdominal portion of the aorta of the second dog showed large fibrous intimal thickenings with scattered foam cells at their bases (fig. 2). The large and small arteries of the first dog were extensively effected by small and large intimal cushions composed of foam cells and of fibrous and hyaline cushions often lined by foam cells (fig. 3). The media beneath such foci as well as remote from them revealed circumscribed medial hyalinizations and calcifications, frequently involving fragmented and swollen elastic membranes (fig. 4).

The internal organs of the 2 dogs which received injections of the low viscous hydroxyethylcellulose exhibited extensive lesions. The liver cells were distended and had sharply defined cellular membranes and foamy cytoplasmic structure. The splenic reticular cells were distinctly proliferated. The renal glomeruli revealed numerous cystically dilated capillary loops containing either a homogeneous grayish matter or endothelial foam cells (fig. 5). The endothelial cells lining the left ventricle of one dog were markedly swollen and cuboidal or columnar in shape, were arranged in several layers and, sometimes, surrounded hyaline material (fig. 6). In both dogs the ascending part of the aorta and the arch showed crowding of endothelial cells, transformation of endothelial cells into foam cells and accumulation of mononuclear cells in hyaline intimal thickenings (fig. 7). Similar lesions were present in rings taken from the thoracic and abdominal portions, where endothelial cells were locally proliferated, of cuboidal shape and stratified in arrangement, forming tongue-like projections or multilayered coatings (fig. 8). In some parts actual cushions had developed, which were composed of foam cells. In both dogs the outer media of one of the large aortic rings showed extensive hyalinizations of the outer and middle portions of the media, which were infiltrated with small round cells. A small artery was completely lined by a thick coat of foam cells.

#### COMMENT

The most pronounced and most prolonged leukopenia followed the intravenous injection of the most viscous type of hydroxyethylcellulose. This observation is in agreement with a similar one made in connection with the testing of 7 types of methyl cellulose of graduated molecular weights and viscosity.<sup>1d</sup> Corresponding relationships were found concerning the degree and the duration of the acceleration of erythrocytic sedimentation, the relative increase in the viscosity of the plasma and

the degree of anemia which developed after single and repeated injections of these substances. It was noted, on the other hand, that the medium viscous hydroxyethylcellulose exerted the most pronounced hemodiluting action; such action was of only moderate degree with the low viscous type and entirely absent with the highly viscous variety. It is doubtful whether or not such a hydrating effect is responsible for the discrepancy repeatedly observed between the number of erythrocytes and the volume of packed blood cells, which was found at several occasions to be abnormally high during the chronic part of the experiment. It is possible that a swelling of the erythrocytes may have been responsible for such a phenomenon. As the average cell size was not determined, this explanation must remain a tentative one.

The histologic observations were to a certain extent unexpected. The practically complete absence of any significant abnormal changes in the organs of the 2 dogs which had received repeated injections of the highly viscous preparation was noted with surprise, especially as these animals had shown the most pronounced and prolonged hematic reactions, indicating that considerable amounts of the injected foreign colloid were present in the blood. It may be recalled in this connection that the dogs which received injections of the two methyl celluloses of highest viscosity exhibited in the internal organs and the arterial vessels anatomic lesions of a lesser degree than those observed in dogs treated with methyl celluloses of lower viscosity. It is not thought that the difference in the absolute amounts of the various hydroxyethylcellulose injected and in the durations of the treatment entirely accounts for these discrepancies, as the more viscous and, therefore, higher macromolecular hydroxyethylcellulose should have been retained more readily than the less viscous and lower macromolecular varieties.

It was found that the 2 dogs which received injections of hydroxyethylcellulose WS-20 exhibited in the internal organs and the arteries the most severe and generalized lesions of the three groups studied. Only the animals of this series showed phenomena related to the storage of the injected matter by the presence of swollen liver cells with foamy cytoplasm and by the accumulation of grayish-stained homogeneous matter in the lumen and endothelial lining of the capillary loops of the renal glomeruli. No such reactions were seen in the dogs of the other series. Foam cells and fibrous cushions and circumscribed hyaline thickenings of the intima, often associated with degenerations and calcifications of the media of the aorta and of large and medium-sized arteries, were seen in the dogs which received injections of hydroxyethylcelluloses of medium and low viscosity. These lesions were definitely more extensive and more widely distributed in the dogs which received the low viscous preparation than in those treated with the medium viscous one. In the former group, moreover, swelling

and proliferation of the endocardial cells were present. It is noteworthy that also this atheromatogenic agent elicited both atheromatous intimal and degenerative and calcifying medial reactions such as those observed after the injection of methyl cellulose. The predominantly fibrous character of some of these lesions indicates a high degree of lability of the foam cell reactions elicited by hydroxyethylcellulose and thus presents an analogy to the early fibrous transformations of the atheromatous changes seen after the injection of pectin.

#### SUMMARY

Hydroxyethylcellulose of three grades of viscosity dissolved in isotonic solution of sodium chloride was injected intravenously into dogs without eliciting any acute and serious untoward reactions.

The most viscous hydroxyethylcellulose caused the most pronounced symptoms (leukopenia, accelerated erythrocytic sedimentation, anemia) characteristic of the macromolecular hematologic symptom complex.

The medium viscous hydroxyethylcellulose produced the most pronounced hemodiluting action.

Storage phenomena in the liver cells and the glomerular endothelial cells, as well as atheromatous and fibrous intimal lesions and medial degenerations and calcifications, were most extensive and most widely spread in the dogs which received injections of solutions of the low viscosity material. Such reactions were entirely absent in the dogs treated with the high viscosity preparation.

# EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

## XIII. Vibratory Lability of Plasma Colloids in Rabbits and in Dogs Following Ingestion of Cholesterol

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**I**N PREVIOUS publications<sup>1</sup> the theory was advanced that local vibratory influences acting on the blood may control to a certain extent the lability of the plasma colloids. This effect in the presence of quantitative disturbances of the cholesterol content of the plasma or of qualitative disturbances of its physicochemical status may cause precipitation of cholesterol at certain parts of the vascular tree where the vibratory factors are most active. This mechanism is believed also to account for the local distribution of atheromatous lesions, which are found at the orifices of the aortic branches, at natural and abnormal narrowings, sacculations and curves of arteries and at areas of normal or pathologic whirl formation and pulsatory impacts of the blood, such as the ascending part of the aorta and the first portion of the coronary arteries.

The studies to be reported were undertaken to obtain additional support for this theory. The vibratory lability of the serum of normal and cholesterol-fed rabbits and dogs was investigated with and without the admixture of various detergents, macromolecular colloidal substances and other agents which exert an influence on the colloidal stability of plasma or which had been successfully used in preventing the development of experimental cholesterol atheromatosis in rabbits.

### EXPERIMENTAL PROCEDURES AND OBSERVATIONS

*Normal Rabbit Serum Plus Macromolecular Substances.*—It was shown in experiments reported during recent years<sup>2</sup> that in dogs and rabbits repeated and prolonged intravenous injections of large amounts of solutions of various hydrophilic carbohydritic colloids (polyvinyl alcohol, methyl cellulose, pectin, sodium cellulose glycolate, hydroxyethylcellulose, acacia) and proteinic colloids (gelatin, ovalbumin) result in specific intimal lesions of foam cell atheromatous or fibrohyaline character, respectively, of the arteries. It was therefore of interest to determine the behavior of these substances when they were mixed with serum

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From the Warner Institute for Therapeutic Research.

1. Hueper, W. C.: (a) Arch. Path. 38:162, 245 and 350, 1944; 39:51, 117 and 187, 1945; (b) this issue, p. 130; (c) Experimental Studies in Cardiovascular Pathology: IV. Experimental Atheromatosis in Macacus Rhesus Monkeys, Am. J. Path., to be published.

2. Hueper, W. C.: (a) Arch. Path. 31:11, 1941; 33:1, 1942; 34:883, 1942; (b) this issue, p. 130; (c) Am. J. Path. 18:895, 1942; 20:737, 1944.

and the mixture exposed to prolonged vibration. For the purpose of the experiment 1 part of a 0.5 per cent solution of pectin in isotonic solution of sodium chloride was mixed with 2, 5, 10, 20, 50, 100 and 200 parts of normal rabbit serum; 2 cc. quantities of these mixtures were then placed in test tubes such as are generally used for the Kahn test, incubated for one hour at 37 C. and then shaken for a period of thirty minutes in a small electric shaker such as is employed for the shaking of blood pipets. The tubes were then examined for floccules.

A similar arrangement was employed in examining the behavior of a 1.5 per cent solution of polyvinyl alcohol, a 5.0 per cent solution of acacia, a 0.25 per cent solution of methyl cellulose (viscosity, 400 centipoises), a 0.075 per cent solution of methyl cellulose (viscosity, 4,000 centipoises) and a 1.5 per cent solution of gelatin. All solutions used had the same viscosity as normal plasma.<sup>2</sup> The results obtained are given in table 1.

TABLE 1.—*Vibratory Lability of Mixtures of Rabbit Serum and Macromolecular Substances*

Substance	Mix-ture	Serum Control	Flocculation Observed in Mixture with Given Ratio of Macromolecular Solution to Serum							
			1:0	1:2	1:5	1:10	1:20	1:50	1:100	1:200
Pectin.....	S	0	0	0	Trace	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Polyvinyl alcohol....	S	0	0	+	+	+	+	+	+	+
	U	0	0	0	0	0	0	0	0	0
Acacia.....	S	0	0	+	+	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Methyl cellulose, 400 centipoises	S	0	0	0	0	0	0	0	0	Trace
	U	0	0	0	0	0	0	0	0	0
Methyl cellulose, 4,000 centipoises	S	0	0	0	0	0	0	0	0	0
	U	0	0	0	0	0	0	0	0	0
Gelatin.....	S	0	Trace	+	+	+	+	+	+	+
	U	0	0	0	0	0	0	0	0	0

S = shaken mixture; U = unshaken control.

Mixtures of solutions of polyvinyl alcohol and serum and of gelatin and serum exhibit under the experimental conditions used marked vibratory lability, evidenced by the appearance of floccules; of the mixtures of serum and solutions of acacia, only those with the higher concentrations of acacia display this quality and these to only a moderate degree; mixtures of serum and solutions of methyl cellulose or solutions of pectin do not form any precipitates, or only minor ones, when shaken.

*Normal Rabbit Serum plus Detergents or Iodides.*—In a second set of experiments the influence of small amounts of detergents and of organic iodides on the vibratory lability of normal serum was tested. The study of these two groups of agents was suggested by the fact that detergents generally increase the dispersion of fatty material in a watery medium and thus accentuate the stability of colloidal emulsions of this type, while iodides have been successfully used in the prevention of experimental cholesterol atheromatosis in rabbits. These agents were used as a 0.1 per cent watery solution and were added to the serum in these proportions: 5, 4, 3, 2, 1 and 0 parts making a total of 5 parts or 2 cc. The two liquids were thoroughly mixed and then shaken for thirty minutes.

The following substances were employed: of detergents, Tergitol Penetrant 08,<sup>3</sup> Tergitol Penetrant 4T, Tergitol Penetrant 7, Tergitol Penetrant 4, dehydrocholic acid, lecithin, Triton K-60,<sup>4</sup> Triton NE, Aerosol OT,<sup>5</sup> cetylpyridinium iodide and sodium hexametaphosphate; of iodides, beta iodopropionic acid, 3,5-*I*-diiodotyrosine, *n*-hexyl iodide and hexamethylenetetramine alliodide. The total amount of the mixture of serum and agent was always 2 cc. An unshaken tube having the same content served as control.

Cetylpyridinium iodide produced a precipitate in both the shaken and the unshaken tube. Sodium hexametaphosphate and lecithin had no demonstrable effect on the colloidal stability of the serum. When Tergitol Penetrants 4, 4T and 7 were used, flocculation occurred in all the tubes, and the flocculation decreased in intensity with the decrease of the concentration of the Tergitols in the mixture. With Tergitol 08 there was flocculation in only the three highest concentrations, while with Aerosol OT this effect was observed in the two highest concentrations. Dehydrocholic acid elicited mild flocculation in the second and third highest concentrations. The unshaken tubes containing these mixtures remained clear. The effect of the iodides tested was in general negative with the exception of the appearance of floccules in the highest concentrations of both 3,5-*I*-diiodotyrosine and *n*-hexyl iodide. The readings in some of the tests on iodides was interfered with by the fact that flocculation appeared in the tubes containing untreated rabbit serum after shaking, indicating that in the blood of the normal rabbit substances occur at times which lower the colloidal stability of the serum in the presence of vibratory influences. It was noted, however, that in these instances there was no flocculation in the tubes containing the iodides in low concentration.

*Normal Rabbit Serum Plus Detergents Plus Macromolecular Substances.*—The observations made in the two experiments recorded suggested a test of the influence which a combination of serum, macromolecular substances and detergents might have on the vibratory lability of such mixtures. For this purpose normal rabbit serum was mixed with solutions of polyvinyl alcohol (1.5 per cent), pectin (0.5 per cent), methyl cellulose of a viscosity of 400 centipoises (0.25 per cent) and methyl cellulose of a viscosity of 4,000 centipoises (0.075 per cent) in a ratio of 1 part of macromolecular colloidal solution to 2, 5, 10 and 20 parts of serum. To 2 cc. of each mixture, 0.1 cc. of a 0.1 per cent watery solution of one or another of various detergents was added. The resulting mixture was shaken for thirty minutes. There were serum controls and macromolecular substance controls, as well as a set of unshaken tubes containing the same materials as were present in the shaken ones. The detergents used were Tergitol Penetrants 4, 4T, 7 and 08, Tritons NE and K-60, Roccal,<sup>6</sup> Aerosol OT, cetylpyridinium iodide, dehydrocholic acid and lecithin.

Tergitol 4, 4T, 7 and 08, Triton K-60, Triton NE, Roccal, Aerosol OT, cetylpyridinium iodide, lecithin and dehydrocholic acid did not elicit any flocculation in serum mixed with methyl cellulose of two viscosities (400 and 4,000 centipoises) and

3. Tergitol Penetrant 08 is sodium sulfate of 2-ethyl hexanol; Tergitol Penetrant 4, sodium sulfate of 2-ethyl hexanol; Tergitol Penetrant 4T, triethanolamine tetradecyl sulfate; Tergitol Penetrant 7, sodium alcohol sulfates 3,9-diethyltridecanol.

4. Triton K-60 is lauryl dimethylbenzyl ammonium chloride; Triton NE, organic polyether alcohol.

5. Aerosol OT is dioctyl ester of sodium sulfosuccinate.

6. Roccal alkyl dimethylbenzyl ammonium chloride.

serum mixed with pectin. Mixtures of serum and polyvinyl alcohol with Aerosol OT, on the other hand, were free from flocculation, while mixtures of serum and polyvinyl alcohol containing, respectively, Tergitol Penetrants 4 and 7, Roccal and Triton K-60 showed flocculation only in a concentration of 10 parts serum and 1 part polyvinyl alcohol. The addition of these detergents evidently had aided in the stabilization of the colloidal polyvinyl alcohol-serum mixtures, reducing their vibratory lability.

A similar stabilizing effect of detergents (Tergitols, lecithin, dehydrocholic acid) was seen in experiments with mixtures of serum and a 5 per cent solution of gelatin observed under identical experimental conditions.

*5. Serum of Cholesterol-Fed Rabbits and Dogs With and Without Detergents, Cyanides, Iodides and Thioureas.*—An investigation of the effect of vibration and of additions of colloid-active agents on the serum of animals receiving considerable amounts of cholesterol with their food represented the next logical step

TABLE 2.—*Total Serum Cholesterol and Cholesterol Ester Values of Rabbits Fed Considerable Amounts of Cholesterol*

Rabbit	Appearance	Total Cholesterol, Mg. per 100 Cc.	Cholesterol Esters, Mg. per 100 Cc.
98.....	Milky	469	245
99.....	Milky	1,081	530
100.....	Milky	428	235
101.....	Milky	1,020	571
102.....	Milky	1,051	632

TABLE 3.—*Flocculation Observed in Shaken Serum of Rabbits Fed Cholesterol in Oil*

Rabbit	Undiluted Serum	Diluted 1:10
98.....	Trace	+
99.....	+	+
100.....	0	0
101.....	Trace	Trace
102.....	+	+

in this study. Detergents, iodides, thiocyanides and thiourea compounds were employed in these tests. The rationale for the use of detergents and iodides has been given. Thiocyanides exert a preventive action on experimental cholesterol atheromatosis, as do iodides, and influence the colloidal dispersion of cholesterol.<sup>7</sup> The testing of thiourea compounds was suggested by the fact that these substances are chemically related to thiocyanides and elicit functional and anatomic hyperactivity of the thyroid gland of a type analogous in many respects to that produced by iodides and thiocyanides.

The hypercholesteremic serums of 5 rabbits which had received daily for several months 0.5 Gm. of cholesterol in 15 cc. of corn oil were used for this purpose. The values of total serum cholesterol and of cholesterol esters at the time of the testing are given in table 2.

Undiluted serum of these rabbits and serum diluted with isotonic solution of sodium chloride at a ratio of 1:10 were shaken for thirty minutes. Unshaken controls remained unchanged. The shaken specimens showed the reactions listed in table 3.

7. Soerensen, S. P. L.: *Kolloid Ztschr.* 53:306, 1930.

In a second set of tubes, to 2 cc. of each of these serums was added 0.1 cc. of a 0.1 per cent watery solution of, respectively, potassium thiocyanide, potassium iodide, lithium iodide, cetylpyridinium iodide, Triton NE, Triton K-60, Aerosol OT and Roccal. The tubes were shaken for thirty minutes. Unshaken tubes with the same contents served as controls. These were always free from flocculation. The results in the shaken tubes are given in table 4.

TABLE 4.—*Flocculation Observed in Shaken Serum of Cholesterolized Rabbits with Additions of Colloid-Active Agents*

Rabbit	Potassium Thiocyanide	Potassium Iodide	Lithium Iodide	Cetylpyridinium Iodide	Triton NE	Triton K-60	Aerosol OT	Roccal
98	+	4+	+	4+	Trace	3+	+	3+
99	3+	3+	2+	+	4+	4+	+	+
100	+	0	3+	Trace	+	3+	Trace	0
101	2+	+	4+	3+	2+	2+	0	Trace
102	2+	4+	2+	2+	Trace	Trace	2+	2+

It is obvious that the addition of the colloid-active agents accentuated in general the vibratory lability of the serum of the cholesterol-fed rabbits. The effect was least pronounced with Aerosol OT.

The fact that the degree of accentuation did not run parallel to the total serum cholesterol level suggested a study of the influence of the concentration of the surface-active agents on the vibratory lability of the lipemic serum. The serum of rabbit 99 was used in subsequent experiments in which 2 cc. of serum was mixed with 0.1 cc. of one or another of the surface-active agents in concentrations of 1:1,000, 1:5,000, 1:10,000, 1:20,000, 1:50,000 and 1:100,000. The following agents were tested: potassium thiocyanide, guanidine thiocyanide, ethylene thiocyanide, lithium iodide, allylthiourea, 5-diethylthiourea, *n*-methyl thiourea and 5-aminouracil. The results are presented in table 5.

TABLE 5.—*Vibratory Lability of Lipemic Rabbit Serum with Additions of Graded Amounts of Colloid-Active Substances*

Substance	Flocculation Observed in Mixture with Given Ratio of Colloid-Active Substance to Serum					
	1:1,000	1:5,000	1:10,000	1:20,000	1:50,000	1:100,000
Potassium thiocyanide.....	+	0	4+	2+	0	+
Ammonium thiocyanide.....	+	0	4+	Trace	0	4+
Guanidine thiocyanide.....	+	Trace	4+	3+	0	2+
Ethylene thiocyanide.....	+	0	4+	+	0	4+
Lithium iodide.....	2+	Trace	4+	2+	0	3+
Allylthiourea.....	0	0	0	2+	Trace	0
Diethylthiourea.....	2+	+	2+	Trace	3+	Trace
<i>n</i> -Methylthiourea.....	+	0	0	0	0	4+
5-Aminouracil.....	Trace	0	0	Trace	2+	3+

The recorded results (table 5) indicate that the various agents used exert a definite effect on the vibratory lability of serum of cholesterolized rabbits. In some concentrations they may stabilize it and in others they may accentuate it. In the case of the thiocyanides these opposite effects seem to alternate with decreasing concentrations in wavelike fashion.

In many of these experiments an additional set of tubes with the same contents as the aforementioned ones was shaken. However, in this set either the

tubes were filled completely, leaving no air space between the cork and the liquid, or the liquid was overlaid with heavy liquid petrolatum, which excluded any contact of the serum mixture with the air and thus any formation of foam. In none of these specially treated tubes was there evidence of flocculation, an observation indicating that contact with air is essential for the production of flocculation.

In one of the experiments with potassium thiocyanide, a chemical analysis was made for the total nitrogen and the total cholesterol content of the supernatant serum and of the floccules obtained as sediment after centrifugation. It was found that the sediment contained about five times as much nitrogen as an equivalent amount (volume) of supernatant serum (5.8 against 28.9 Gm. of protein per hundred cubic centimeters) and that the sediment contained more than twice as much cholesterol as the supernatant serum (320 mg. against 750 mg. per hundred cubic centimeters). This observation suggested that the floccules were composed of a mixture or condensation product of proteins and cholesterol.

In a final experiment tests were made of the vibratory lability of the serum of 4 normal stock dogs, of 2 dogs which had received daily for four months 5 Gm. of cholesterol plus 5 Gm. of dried total ox bile in 120 cc. of corn oil and of 2 dogs which had been fed daily for three months 5 Gm. of cholesterol plus 15 Gm. of lecithin in 120 cc. of corn oil. Undiluted serum was used and serum diluted with saline solution in ratios of 1:1, 1:2, 1:4 and 1:10, as well as diluted with equal parts of a watery solution of potassium thiocyanide in concentrations of 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, 0.00025, 0.001, 0.0005, 0.00025 and 0.0001 per cent. Total serum cholesterol and ester cholesterol were determined at the same time in the 4 cholesterol-fed dogs: In the cholesterol-bile dogs, the total serum cholesterol was 246 mg. (dog 1938) and 342 mg. (dog 1947) per hundred cubic centimeters, while the ester cholesterol was 57 mg. (dog 1938) and 61 mg. (dog 1947) per hundred cubic centimeters. In the cholesterol-lecithin dogs, the total serum cholesterol was 124 mg. (dog 1878) and 118 mg. (dog 1994) per hundred cubic centimeters, while the ester cholesterol was 50 mg. (dog 1978) and 54 mg. (dog 1994) per hundred cubic centimeters.

After shaking 2 cc. of the serum or of the various mixtures, there were no changes in the tubes containing the serum of the 4 normal dogs. Mild flocculation was found in the cholesterol-lecithin serum containing additions of potassium thiocyanide solution in concentrations upward of 0.025 per cent. The cholesterol-bile serum, on the other hand, exhibited flocculation not only in the higher saline dilutions (1:3 and upward in dog 1947; 1:10 in dog 1938) but also in the tubes containing additions of solutions of potassium thiocyanide. The flocculation was of mild to moderate degree in the serum of dog 1938 and was found with potassium thiocyanide concentrations of 0.5 to 0.005 per cent. In the tubes containing the serum of dog 1947, which had the highest serum cholesterol content, the precipitation started at a potassium thiocyanide concentration of 0.25 per cent, increased in severity in the subsequent lower concentrations, reaching a peak between concentrations of 0.025 and 0.0025 per cent and decreasing progressively in intensity toward the lowest concentration, where it was of minimal extent.

The observations reported indicate that both the cholesterol content of the serum and the concentration of potassium thiocyanide exert a definite influence on the vibratory lability of the serum colloids, including the colloidally dispersed cholesterol.

*Chemical Studies of the Blood of Dogs Fed Cholesterol.*—In attempts to obtain additional information on the physicochemical changes which apparently

take place in the plasma of animals fed a high cholesterol-fat diet, chemical studies of the serum of dogs were made to determine the immediate effects on the various lipid fractions of the serum of meals containing cholesterol in oil either alone or in combination with protein (100 Gm. of beef or fish), lecithin (5 Gm. and 15 Gm.) or dried bile (5 Gm.). Blood was withdrawn from the jugular vein three, four, five, seven, twenty-four, twenty-eight and forty-eight hours after such a meal for this purpose. Eighteen dogs were used in the investigation, and 38 tests were made on them.

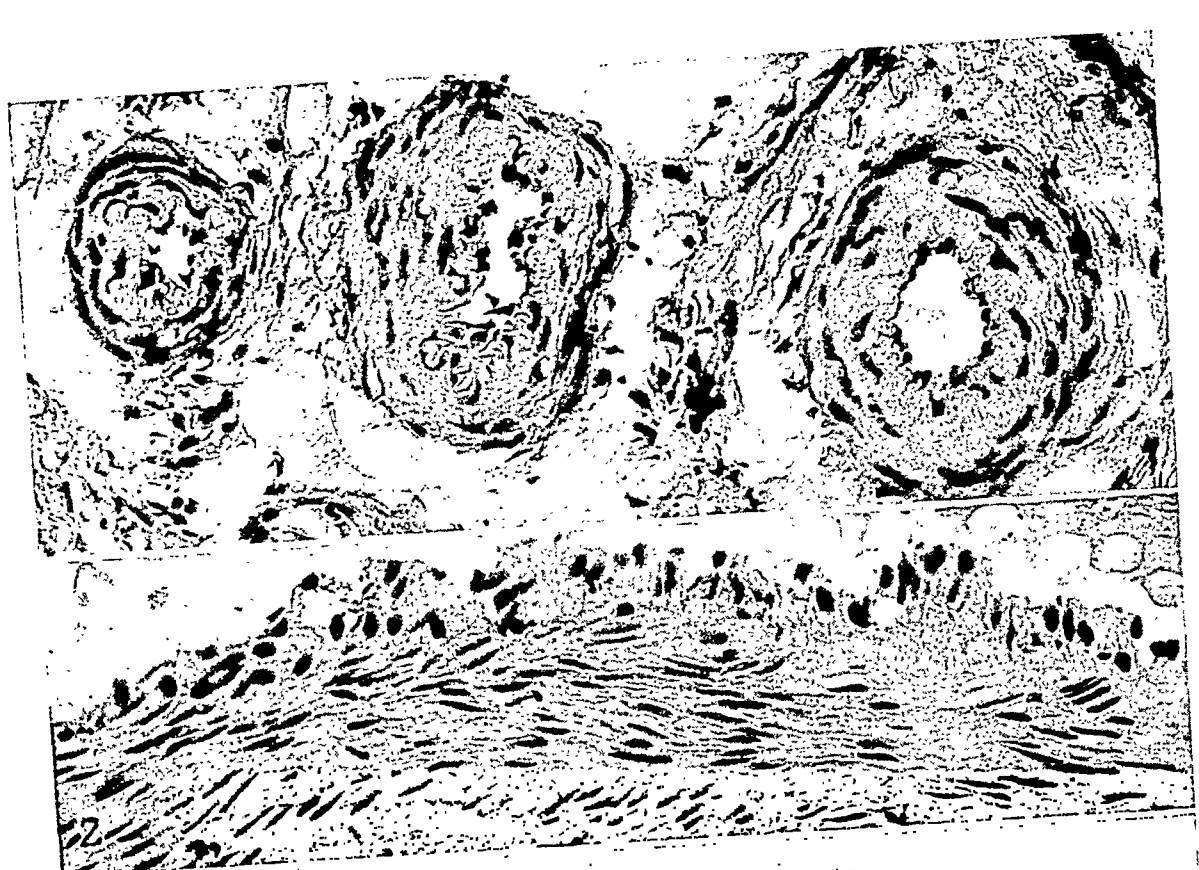
In 8 of the 18 dogs a prolonged course of cholesterol in oil feeding was subsequently undertaken. Four of these dogs received 5 Gm. of cholesterol plus 5 Gm. of lecithin dissolved in 120 cc. of corn oil mixed with Purina dog chow<sup>8</sup> every other day in addition to their ordinary ration of dog chow. Two were fed this diet for five months and the other 2 for six months. All were then killed by an intravenous injection of a 4 per cent formaldehyde solution. Two dogs received every day for three months 5 Gm. of cholesterol plus 15 Gm. of lecithin in 120 cc. of corn oil. Two dogs were fed daily for three months 7 Gm. of cholesterol plus 7 Gm. of dried ox bile dissolved in 120 cc. of corn oil. The blood of the dogs of the first group was studied every two weeks, while that of the dogs of the second and third groups was studied only during the first month and again directly before they were killed.

The following items were determined: Total serum cholesterol, total ether-extractable cholesterol, ester cholesterol, ether-extractable ester cholesterol, fatty acids, phospholipids, in some instances also total serum calcium, ionizable calcium and nonprotein nitrogen. Hematologic studies were conducted at the same time covering the following items: hemoglobin, erythrocytes, leukocytes, erythrocytic fragility, coagulation time, prothrombin time, sedimentation rate, packed red cell volume, vibratory serum lability and appearance.

A Lumetron photocolorimeter was used for the chemical studies of the blood, with the methods adapted for this apparatus. In the preparation of the ether extracts of total and ester cholesterol 1 cc. of serum was shaken in a 25 cc. volumetric flask with 15 cc. of ether. The content of the flask was then brought to a boil and allowed to boil for a few seconds. After it had cooled and been diluted to make 25 cc., 9.5 cc. of it was used for the determination of total cholesterol and 14.5 cc. for that of ester cholesterol, the digitonin method being employed.

(a) Hematic Observations: The serum was milky after a cholesterol-oil meal for a period of up to twenty-eight hours, the peak of turbidity being shown at four to six hours after ingestion. In the majority of dogs there was a reduction of the number of erythrocytes during the first twenty-four hours after the test meal, but in only 4 of them did the number drop by more than 1,000,000 cells. The amount of hemoglobin and the volume of packed red cells underwent fluctuations paralleling those of the erythrocytes. While the fragility tests revealed variations in the osmotic resistance of the erythrocytes, these reactions remained within normal limits and showed no relation to the fluctuations in the number of red cells. The number of leukocytes, the prothrombin time and the sedimentation rate did not exhibit any significant changes. The coagulation time, on

8. According to the Ralston Purina Company, the ingredients of the chow are as follows: meat meal, dried skim milk, riboflavin, carotene, cod liver oil, brewers' dried yeast, wheat germ, corn grits, wheat cereal, corn cereal, dried beet pulp, molasses, steamed bone meal and iodized salt. The chemical analysis shows: protein, 23 per cent, fat 5 per cent, fiber 4 per cent, ash 7 per cent, nitrogen-free extract 54 per cent and moisture 7 per cent.



(See legend on opposite page)

the degree of colloidal instability of the serum cholesterol as represented by the ether-extractable fraction has some connection with the lipemic character of the serum after the ingestion of cholesterol in oil. However, it is apparent that other factors must play a role in the production of this phenomenon.

*Anatomic Observations.*—The 8 dogs which were subjected to prolonged feeding with the various cholesterol-oil mixtures and which were killed at the end of the experimental period by an intravenous injection of a 4 per cent formaldehyde solution were examined post mortem. There were no significant macroscopic changes of the internal organs with the exception of general obesity, especially characterized by large amounts of subcutaneous and retroperitoneal fat tissue. The liver of 1 dog had a brown-yellow color. Six of the dogs were 1½ to 2½ years old; the seventh was 6 months old and the eighth 4½ years old.

A histologic examination was made of each of the following organs: brain, hypophysis, thyroid and parathyroid glands, lung, heart, liver, pancreas, spleen, adrenal gland, kidney, testis, uterus, vena cava inferior, thymus, duodenum, bone marrow (sternum), aorta, brachial arteries, carotid arteries, iliac arteries and various small branches of the abdominal aorta. For microscopic study the vessels were cut into numerous narrow rings.

With the exception of the acute circulatory reactions elicited by the injection of the formaldehyde solution, the internal organs were essentially free from abnormal changes attributable to the dietary management with cholesterol. One of the dogs which were fed cholesterol plus dried bile exhibited a severe fatty infiltration of the liver cells. The medium-sized intrarenal vessels of this animal showed local medial hyalinizations (fig. 1). The aortas and arteries of the 4 dogs fed cholesterol and lecithin in oil were entirely free from any atheromatous or foam cell lesions with the possible exception of a dog which revealed small foci of proliferated and swollen endothelial cells in the pulmonary artery (fig. 2). There were only calcified hyaline areas in the media of the aorta at the distal end of the aortic bulb. In addition to a similar lesion of the aorta, in 1 of the 2 dogs which received cholesterol in oil there was a small focus in the ascending part of the aorta in which the endothelial cells were swollen and cuboidal or columnar and lined the aortic wall in a stratified or palisade-like arrangement (fig. 3). The vena cava inferior of this animal showed hyaline intimal thickening and calcification of the elastic membrane. There were no abnormal alterations in the vessels of the second dog belonging to this series.

The aorta of 1 of the 2 dogs fed cholesterol plus bile exhibited in its ascending part subintimal hyalinizations of the media with small scattered deposits of blue-stained calcium granules embedded in the hyaline matter. The vena cava inferior of this dog had in places an endothelial lining which was stratified or formed tonguelike processes consisting of swollen cuboidal cells (fig. 4). The ascending part of the aorta of the second dog also revealed local medial hyalini-

#### EXPLANATION OF PLATE.

Fig. 1.—Small renal arteries with thickened and locally hyalinized walls.

Fig. 2.—Pulmonary artery with proliferated and swollen endothelial cells.

Fig. 3.—Cuboidal and columnar endothelial cells forming small cushions in the aorta.

Fig. 4.—Vena cava inferior with tonguelike proliferations of swollen endothelial cells.

zations with bluish granular deposits. Here, as well as in other rings, there were areas composed of stratified arrangements of swollen cuboidal or columnar endothelial cells forming pointed processes.

#### COMMENT

The analysis of the various observations made in these experimental studies reveals the fact that the conditions controlling the vibratory lability of the plasma are highly complex. It was shown that numerous factors may cause the appearance of floccules in serum exposed to shaking, while others counteract such precipitations. The basic fact, however, is apparent, that the development of such precipitates can be elicited only when the surface of the serum mixtures is exposed to air. Thus the production of foam and thereby the formation of film are permitted. When this mechanism is excluded by complete filling and stoppering of the tubes or by overlaying of the liquid content with heavy liquid petrolatum flocculation is consistently prevented. It was observed that the precipitates developed not only in serums to which macromolecular substances or colloidally active agents were added but also in serums of animals showing dietary hypercholesterolemia and occasionally also in serums of "normal" animals.

The ease with which floccules appear in the serum of animals given injections of solutions of polyvinyl alcohol or gelatin has been previously noted incidental to *in vivo* experiments<sup>9</sup> with other agents. The present *in vitro* investigations confirm those observations. It is remarkable, on the other hand, that other atheromatogenic macromolecular substances, such as methyl cellulose and pectin, did not respond with the formation of floccules when shaken with normal rabbit serum. It was observed, however, that the serum of hypercholesteremic rabbits and of dogs fed large amounts of cholesterol showed floccular precipitates on shaking. Thus these observations support only in part the theory of the significance of vibratory influences in the production of atheromatous lesions. The absence of air in the blood circulating in the vascular tree eliminates the important factor of film formation in the interface for the production of precipitates *in vivo*. These consist of cholesterol-protein complexes.

The various detergents tested apparently increased the vibratory lability of normal serum. The iodides, on the other hand, were in general without any definite effect in this respect. When added to mixtures of serum and solutions of macromolecular colloids some of the detergents (Tergitol Penetrants 4 and 7, Roccal and Triton K-60) seemed to exert a stabilizing influence on the mixtures previously found to be relatively unstable (serum plus polyvinyl alcohol, serum plus

9. Footnotes 1a and 2c.

gelatin). The type of detergent, as well as the colloidal medium, thus plays an important role in determining the effect of vibratory influences on colloidal stability. Studies on hypercholesteremic serum showed that the concentration of the colloid-active agents (thiocyanides, thioureas) is also a deciding factor in this respect, as the colloidal vibratory lability of such serum was either intensified or diminished, depending on the concentration of the various thiocyanides and thioureas added. The present investigations, however, are not sufficiently elaborate for deciding whether or not variations in the status of the plasma colloids are reflected by definite patterns of colloidal vibratory lability having diagnostic significance.

The positive findings in the serum of hyperlipemic rabbits and in the serum of dogs with quantitatively normal total cholesterol and ester cholesterol which ingested large amounts of cholesterol in oil indicate that not only an excessive amount of cholesterol in the serum but also the physicochemical status of the serum cholesterol as influenced by dietary intake plays a decisive role in the vibratory lability of this colloidal liquid. The frequent and considerable increase of the ether-extractable fraction of free and of bound cholesterol observed in dogs which had ingested cholestrol in oil suggests that this factor may be important in this respect, as the ether-extractable portion of cholesterol is that fraction which is not bound in some way to the plasma proteins and which is, thus, colloidally not stabilized (Soerensen<sup>7</sup>; Handovsky<sup>10</sup>). A relative increase of the ether-extractable fraction of serum cholesterol, therefore, may indicate an elevation of a colloidally labile fraction of cholesterol, which may occur in the absence of any significant and abnormal rise in the levels of total and ester serum cholesterol. It seems to be possible that such shifts in the physicochemical status of cholesterol in the serum under the influence of dietary factors are responsible for the appearance of floccules in the serum that is exposed to shaking and may be of significance for the production of dietary atheromatosis.

If this phenomenon may be taken as an index of increased vibratory colloidal lability of the serum, it may provide a plausible explanation for the appearance of the localized proliferations of swollen endothelial cells often forming cellular processes and coatings in the aorta, the pulmonary artery and the vena cava inferior of several of the dogs kept for several months on a high cholesterol diet. These lesions appear to be early atheromatous manifestations, as they resemble those seen in rabbits after cholesterol feeding as well as those observed in dogs given injections of various macromolecular substances, particularly hydroxyethylcellulose.<sup>11</sup> They were most marked in the dogs which received

10. Handovsky, H.: *Klin. Wehnschr.* **3**:1354, 1924.

for only three months 7 Gm. of cholesterol plus 7 Gm. of dried ox bile in 120 cc. of corn oil, while they were absent in all but 1 of the dogs which received a cholesterol-lecithin-oil mixture.

These observations are in general agreement with those made recently by Member, Bruger and Oppenheim,<sup>11</sup> who found that the combined feeding of cholesterol and bile acids to rabbits favored the development of a high cholesterol content of the blood and of the aorta and aortic atheromatosis. Steiner and Domanski,<sup>12a</sup> on the other hand, were unsuccessful in eliciting in dogs kept for forty-four to fifty-six weeks on a diet containing 100 Gm. of egg yolk powder daily any atheromatous arterial lesions. The egg yolk powder consisted of lecithin 14.4 per cent, cholesterol 8 per cent and fat 40.6 per cent. Experiments of the same investigators have shown that the administration of soybean lecithin to patients caused hypocholesterolemia, while Kesten and Sibowitz<sup>13</sup> reported that in rabbits receiving soybean lecithin together with cholesterol only minimal or no atheromatosis developed. This anti-atheromatogenic effect of lecithin may be due to the fact that lecithin tends to keep cholesterol in the dispersed phase and thus counteracts its flocculation and deposition.

Mention may be made in this connection of the observations of Frey<sup>14</sup> concerning shifts in the globulin-albumin ratio in the tissues of the vascular walls preceding the deposition of cholesterol and calcium and their influence on this phenomenon through the resulting disturbance of the colloid equilibrium. Similar factors are apparently responsible for the reduced stability of cholesterol in the serum of persons with atherosclerosis. Frey found that when 0.1 Gm. of cholesterol is added to 5 cc. of serum obtained from normal persons, there is usually a distinct increase in the cholesterol content of the serum. When, on the other hand, the serum of atherosclerotic persons is used, there occurs a definite decrease in the cholesterol content of the serum as cholesterol is precipitated from the serum. Such reductions may amount to more than 100 mg. of cholesterol per hundred cubic centimeters of serum and indicate that the serum of atherosclerotic persons contains a labile fraction of cholesterol.

Mention may be made finally of some of the hematic reactions observed in the dogs kept on a cholesterol diet. The transitory decreases

11. Member, S.; Bruger, M., and Oppenheim, E.: Arch. Path. **38**:210, 1944.

12. Steiner, A., and Domanski, B.: (a) Am. J. M. Sc. **201**:820, 1941; (b) Proc. Soc. Exper. Biol. & Med. **55**:236, 1944.

13. Kesten, H. D., and Sibowitz, R.: Proc. Soc. Exper. Biol. & Med. **49**: 71, 1942.

14. Frey, W.: Verhandl. d. deutsch. Gesellsch. f. inn. Med. **51**:51, 1939.

in the number of erythrocytes found in the majority of the dogs after a single meal with added cholesterol in oil are possibly attributable to the massive influx of fatty acids into the blood, causing there increased osmotic fragility (Johnson, Freeman and Longini<sup>15</sup>; Davis<sup>16</sup>; Johnson, Freeman, Longini and Loewy<sup>17</sup>; Longini and Johnson<sup>18</sup>), an effect which was not unequivocally demonstrable in the present experiments. It is possible that such a destructive action on the erythrocytes may be the cause of the simultaneous considerable shortening of the coagulation time observed. The significant increase in the number of erythrocytes found in 2 of the 4 dogs subjected to prolonged administration of cholesterol and lecithin in oil may represent a phenomenon compensatory for the increased erythrocytic destruction causing overstimulation of the bone marrow, or may be the result of anoxia of the marrow resulting from the circulatory effect of the choline fraction contained in lecithin, as proposed by Davis.<sup>16</sup> The elevation of the prothrombin time observed in all dogs of this series, on the other hand, may be related to functional reactions of the liver brought about by the abnormal intake and the hepatic metabolism of the various lipids.

#### SUMMARY

Floccules appear in normal rabbit serum which has been mixed with a solution of polyvinyl alcohol and a solution of gelatin, on shaking. Identical reactions occur when the hyperlipemic serum of rabbits fed cholesterol and the normally lipemic serum of dogs fed cholesterol are shaken with solutions of various colloid-active substances (thiocyanides, thioureas) or are diluted with isotonic solution of sodium chloride. Detergents added to normal rabbit serum tend to increase the vibratory lability and often elicit formation of floccules composed of protein-cholesterol complexes. Certain detergents, on the other hand, when added to mixtures of serum and polyvinyl alcohol or gelatin exert a stabilizing effect on the colloidal equilibrium of the mixtures. The vibratory lability of hypercholesteremic rabbit serum is either intensified or diminished by the addition of thiocyanides and thioureas, depending on the relative concentrations of these agents. The development of a precipitate is bound to the formation of a film in the foam produced by shaking in the interface of air and liquid. Dogs fed considerable amounts of cholesterol in oil show a definite increase of the ether-

15. Johnson, V.; Freeman, L. W., and Longini, J.: *J. A. M. A.* **124**:1250, 1944.

16. Davis, J. E.: *Am. J. Physiol.* **142**:65, 1944.

17. Johnson, V.; Freeman, L. W.; Longini, J., and Loewy, A.: *Federation Proc.* **3**:22, 1944.

18. Longini, J., and Johnson, V.: *Am. J. Physiol.* **140**:349, 1943.

extractable fractions of total cholesterol and ester cholesterol, which are probably colloidally less stable than the cholesterol fractions bound to proteins.

Several of the dogs, especially those receiving cholesterol in oil plus dried ox bile dissolved in corn oil, showed localized proliferations and swelling of the endothelial lining of the aorta, the pulmonary artery and the vena cava inferior. These lesions apparently represent early atheromatous changes.

# STRUCTURAL CHANGES IN THE THYROID GLANDS OF PATIENTS TREATED WITH THIOURACIL.

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THE morphologic changes in the thyroid glands of patients with hyperthyroidism are well enough known so that a fair correlation can be made between structure and function. This situation seemingly ended abruptly when thiouracil was introduced for the treatment of hyperthyroidism.<sup>1</sup> Clinical observations on patients with hyperthyroidism treated with thiouracil are accumulating. Morphologic studies of the thyroid glands of such patients, however, are comparatively few.<sup>2</sup> Yet such studies may shed considerable light on the probable mechanism of, and the principles involved in, the action of thiouracil. In this paper we present the results of observations on 7 patients with marked signs and symptoms of exophthalmic goiter who were so treated as to provide material for a study of the effects on the thyroid gland of thiouracil alone, of strong solution of iodine U.S.P. followed by thiouracil, and of thiouracil followed by strong solution of iodine U.S.P.<sup>2a</sup>

## PATIENTS TREATED WITH THIOURACIL ONLY

CASE 1.—E. K., a 48 year old woman, was admitted to the University Hospitals on Sept. 4, 1944. Nine months previously she had noted rapid loss of weight and increased appetite, with subsequent development of nervousness, tremor, excessive perspiration, tachycardia and fatigability. Within six months she had lost 40 pounds (18 Kg.) and was bedfast most of the time due to extreme weakness. On admission, she was extremely nervous, weighed 98 pounds

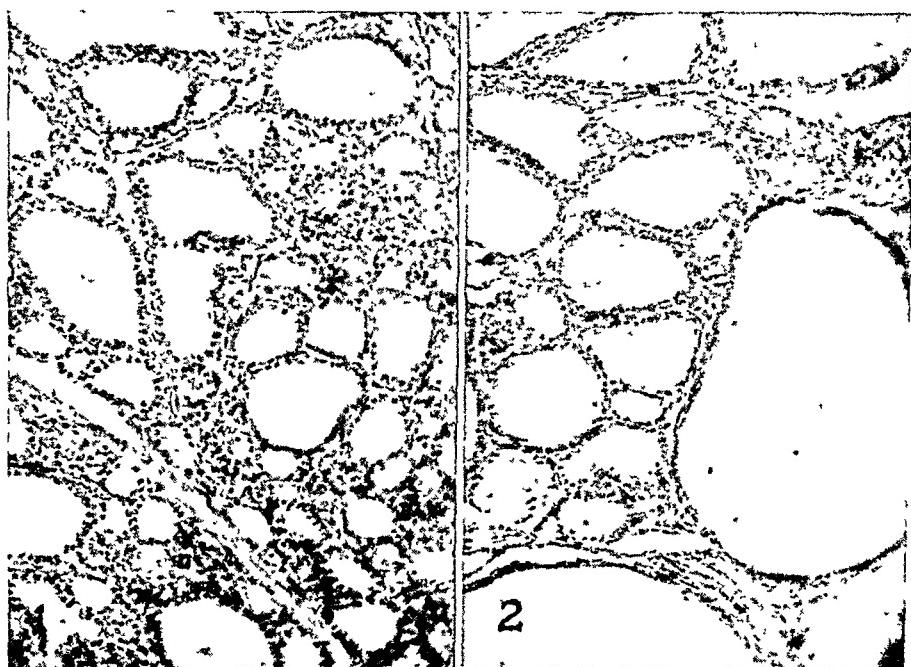
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From the Departments of Pathology, Surgery and Medicine, University of Oklahoma School of Medicine.

1. Astwood, E. B.: J. A. M. A. **122**:78, 1943.
2. Williams, R. H., and Clute, H. M.: New England J. Med. **230**:657, 1944. Rawson, R. W.; Evans, R. D.; Means, J. H.; Peacock, W. C.; Lerman, J., and Cortell, R. E.: J. Clin. Endocrinol. **4**:1, 1944. Moore, F. D.; Sweeney, D. N., Jr.; Cope, O.; Rawson, R. W., and Means, J. H.; Ann. Surg. **120**:152, 1944. Lozinski, E., and Siminovitch, J.; Canad. M. A. J. **51**:422, 1944. Palmer, M. V.: Ann. Int. Med. **22**:335, 1945.

- 2a. The thiouracil used in these studies was supplied by the Medical Research Department of the Winthrop Chemical Company, Inc.

(44.5 Kg.), presented a moderately diffuse enlargement of the thyroid gland and a fine tremor and had slight exophthalmos. The pulse rate was 108; the blood pressure was 145 systolic and 76 diastolic, and the basal metabolic rate was plus 58 per cent. Thiouracil was administered orally, 0.6 Gm. daily in divided doses, for three weeks. At the end of this time the basal metabolic rate was plus 25 per cent, the pulse rate 84, and the blood pressure 126 systolic and 72 diastolic. Thiouracil was not available for the next two weeks, during which time the basal metabolic rate rose to plus 58 per cent. Four weeks after thiouracil therapy was resumed (0.4 Gm. daily), the basal metabolic rate was plus 12 per cent, the pulse rate 88, and the blood pressure 130 systolic and 68 diastolic. She had gained only 1 pound. Eight weeks after the beginning of thiouracil therapy (on November 1) subtotal thyroidectomy was performed by Dr. John W. Cavanaugh.



Figs. 1 and 2 (cases 1 and 2).—Microscopic appearance of the thyroid glands of patients treated with thiouracil only. Various-sized acini are seen lined by cuboidal or low columnar cells, with the lumens containing faintly stained colloid, vacuolated and scalloped.  $\times 100$ .

*Description of Specimen.*—The thyroid tissue removed comprised both lobes, measuring 6 by 4 by 2.5 and 5.5 by 3.5 by 2 cm. The external surfaces were coarsely lobulated. The posterior surfaces were ragged and had a colloid sheen. On microscopic examination (fig. 1) acini of variable size lined by cuboidal or low columnar cells were seen fairly close to one another, separated by delicate or incomplete septums. The lumens contained faintly stained colloid, vacuolated and scalloped, or were empty. Groups of interacinous cells were numerous in some of the lobules.

**CASE 2.**—Z. L., a 44 year old Negro housewife, was first admitted to the University Hospitals March 20, 1944. She complained of nervousness and loss of weight with increased appetite. She had had a goiter for five years. Seven months prior to admission she noticed an increase in the size of her neck and

progressive bulging of her eyes. She denied having taken iodine at any time. On admission, the thyroid gland was diffusely enlarged, and she had well developed signs and symptoms of exophthalmic goiter. Her weight was 122 pounds (55 Kg.). The blood pressure was 160 systolic and 65 diastolic, and the heart rate was 144. A mitral and aortic systolic murmur could be heard, which was transmitted to the vessels of the neck and the axilla. The basal metabolic rate was plus 60 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses, beginning March 25, when her weight was 118 pounds (53.5 Kg.). Forty-nine days later her weight had increased from 118 to 128 pounds (53.5 to 58 Kg.), and the basal metabolic rate was plus 13 per cent, the blood pressure was 160 systolic and 90 diastolic, and the pulse rate was 84. On May 16 bilateral subtotal thyroidectomy was performed by Dr. John H. Robinson.

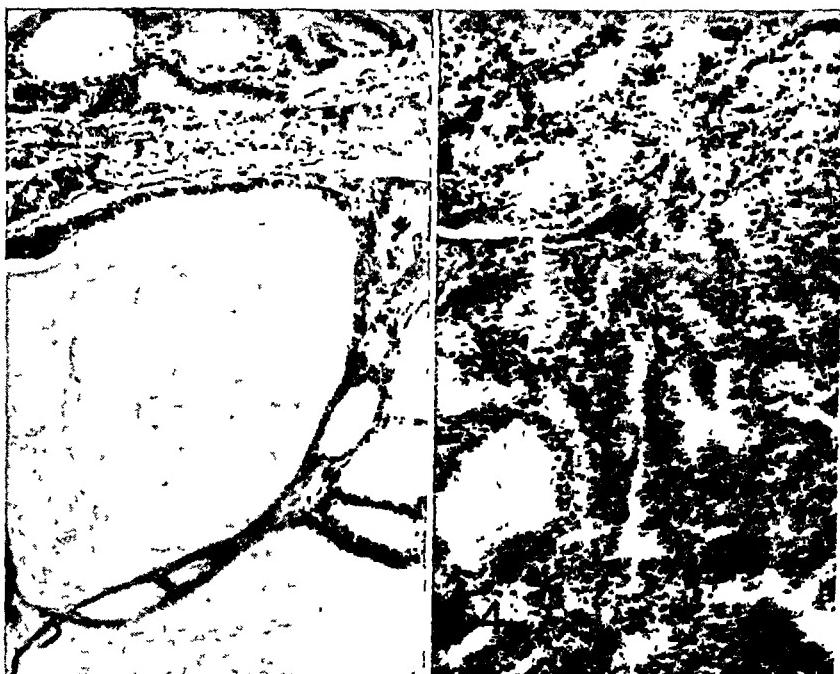
*Description of Specimen.*—The thyroid tissue removed consisted of both lobes, measuring 4.5 by 3.5 by 3 and 4.5 by 3.5 by 2.5 cm. On each lobe there was a ragged surface, measuring 4 by 2 cm.; the remaining surfaces were nodular and covered with a thin, transparent membrane. The cut surfaces were gray-pink and lobulated, with delicate septums separating the lobules. At one pole there was a circumscribed nodule, 1.5 cm. in diameter, which had a complete capsule. The nodule was pale yellow-pink and had a colloid sheen. On microscopic examination (fig. 2) variable-sized acini were seen in groups, some covering the greater part of a microscopic field. The acini were separated by delicate or incomplete septums, which were lined by flat or cuboidal cells. In other fields the acini were small and were lined by cuboidal or columnar cells and separated by sheets of interacinous cells. Some of the acini contained a light-stained, vacuolated and scalloped colloid. Occasional ones were empty. Spur-like protrusions covered by columnar cells were seen in some of the lumens. In the stroma there were aggregations of lymphocytes, plasma cells and large mononuclear cells.

#### PATIENTS TREATED WITH STRONG SOLUTION OF IODINE U. S. P. FOLLOWED BY THIOURACIL

CASE 3.—M. H., a 33 year old housewife, was admitted to the University Hospitals March 14, 1943. She had had a goiter for fifteen years, and because of symptoms of hyperthyroidism she had been treated intermittently with strong solution of iodine U. S. P. until about one and one-half years before her admission. At that time the basal metabolic rate was plus 43 per cent. After preparation with strong solution of iodine, on April 7, bilateral subtotal thyroidectomy was performed by Dr. Charles M. O'Leary. When discharged the patient was given strong solution of iodine and instructed to return in four weeks. She failed to return, continued to have symptoms of hyperthyroidism and continued taking strong solution of iodine until her readmission on Feb. 11, 1944. At this time there were recurrent enlargement of the thyroid gland, nervousness, palpitation, marked exophthalmos with pain in the eyes, loss of weight and weakness. She weighed 147 pounds (66.5 Kg.), her pulse rate was 120 and the basal metabolic rate was plus 28 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses. Eighteen days later the basal metabolic rate had dropped to plus 9 per cent, and she was discharged from the hospital with instructions to take 0.2 Gm. of thiouracil daily. She continued taking the drug until her third admission May 29, when she weighed 167 pounds (75.5 Kg.) and the basal metabolic rate was minus 26 per cent. On June 2, bilateral subtotal thyroidectomy was performed by Dr. Charles M. O'Leary.

*Description of Specimen.*—The specimen obtained at the first operation (April 7, 1943) consisted of two pieces of thyroid tissue measuring 7 by 4 by 2.5 and 6.5 by 4 by 2 cm. The surfaces were brown-pink, smooth and glistening. The cut surfaces were pink-red, with a meaty appearance and a colloid sheen. On microscopic examination (fig. 3) there were marked variations in the size of the acini, which were lined by flat or low cuboidal cells. Some had infoldings covered with columnar epithelium. The colloid stained uniformly pink. In some areas the septums were delicate and in places incomplete. In other areas there were acini with small lumens and groups of interacinous cells. The septums in places were infiltrated with lymphocytes to a degree almost amounting to lymph follicle formation.

The specimen obtained at the second operation (June 2, 1944) consisted of three globular masses of thyroid tissue measuring 5 by 4 by 3 and 5 by 3.5 by 2.5 and 5 by 2.5 by 2 cm., respectively. One surface of each was raw; the other was



Figs. 3 and 4 (case 3).—Microscopic appearance of the thyroid gland of a patient treated with strong solution of iodine U. S. P. followed by thiouracil. The patient was a 33 year old woman, with a basal metabolic rate of minus 26 per cent at the time of her second operation (fig. 4).  $\times 100$ .

covered by a delicate membrane. The cut surfaces were pale gray, with no colloid sheen. On microscopic examination various-sized acini, some with elongated, others with round lumens, were seen in a scanty connective tissue stroma (fig. 4). The lumens were empty or contained a slight amount of light-stained colloid. The lining cells were tall columnar or cuboidal. The cytoplasm of many of the cells appeared vacuolated. Sheets of interacinous cells were seen with no lumens. Broad bands of hyalinizing fibrous connective tissue subdivided the lobules. Into some of the lumens protruded infoldings lined by tall columnar cells. In places the cell nuclei were heaped up into several rows. In the larger spaces colloid was more abundant and appeared scalloped and vacuolated.

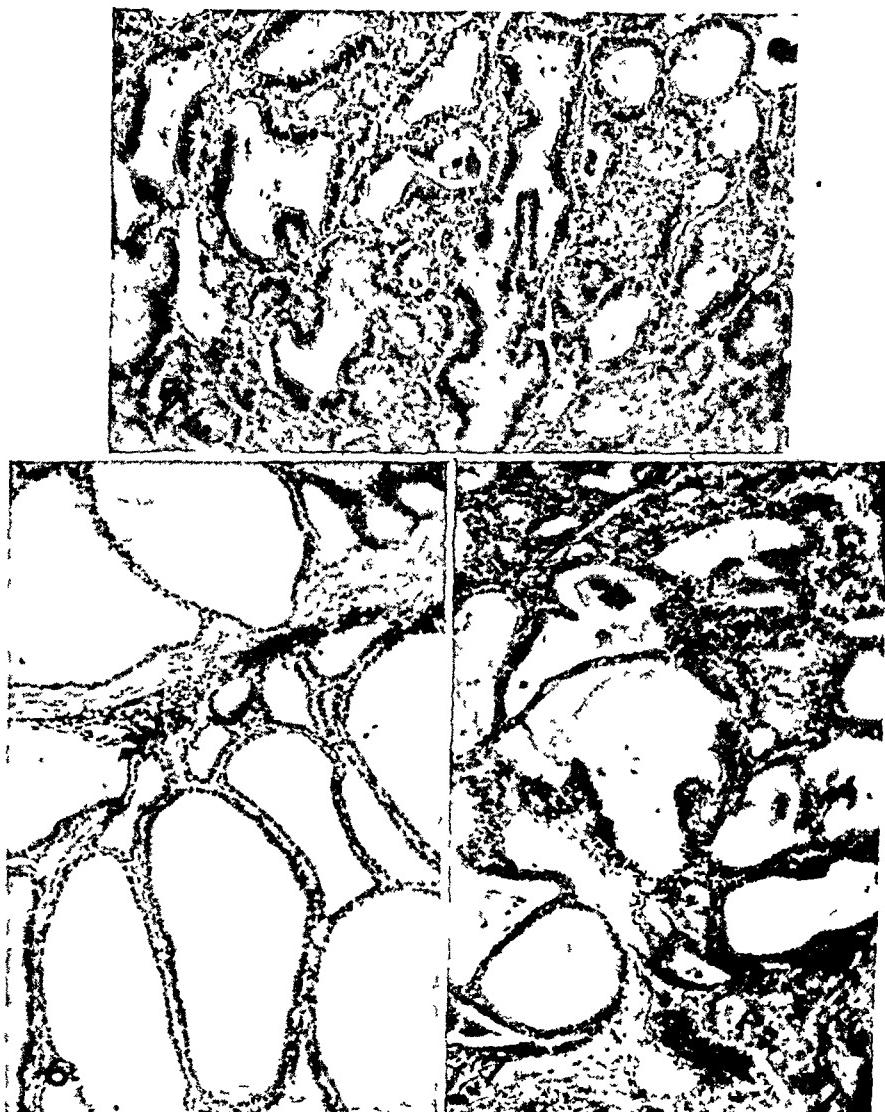
CASE 4.—E. B. S., a 31 year old housewife, was admitted to the University Hospitals March 3, 1944. She had had enlargement of the thyroid gland for twelve years. In 1938 nervousness, loss of weight with increased appetite, tachycardia, intolerance of heat and severe exophthalmos developed. The same year, during a seven month stay in the hospital and after preparation with iodine, the right lobe of the thyroid gland was removed. Improvement was noted until 1940, when her symptoms recurred. Iodine gave some relief for the following year; then objective and subjective symptoms became progressively worse, and for three months prior to admission the symptoms of cardiac decompensation were severe. On admission, the patient's thyroid gland was diffusely enlarged, and she exhibited extreme nervousness, tremor and marked exophthalmos. The heart was enlarged; the pulse rate was 112, the blood pressure was 155 systolic and 55 diastolic. Rales were present in the bases of both lungs, and dependent edema was apparent. She weighed 84 pounds (38 Kg.). Thiouracil was administered, 1 Gm. daily in divided doses, starting March 8, 1944, when the basal metabolic rate was plus 107 per cent. On March 17 the basal metabolic rate was plus 76 per cent, and she had gained 5 pounds (about 2 Kg.). The dose of thiouracil was then reduced to 0.6 Gm. daily. On April 10 her weight was 109 pounds (49.5 Kg.), the basal metabolic rate was plus 29 per cent, the blood pressure was 140 systolic and 80 diastolic and the pulse rate 80. On April 17 bilateral subtotal thyroidectomy was performed by Dr. L. J. Starry.

*Description of Specimen.*—The specimen consisted of one lobe of thyroid gland, measuring 8 by 5 by 3 cm. Its surface was coarsely lobulated and covered by a delicate membrane. One fourth of the surface was raw. The cut surfaces were divided into various-sized gray-pink fields without a colloid sheen. There were four additional portions of thyroid tissue, together measuring 5 by 4 by 2 cm. They resembled the other externally and on their cut surfaces. On microscopic examination (fig. 5) there was marked variation in the size of the acini. Some were elongated and had spurlike infoldings lined by tall columnar cells. The acini contained a light-stained network of fibrillar material with pink-stained colloid, scalloped in places and vacuolated in others. Groups of inter-acinous cells were seen here and there. The connective tissue stroma was scanty, and in places there were aggregations of lymphocytes, amounting to lymph follicle formation.

CASE 5.—G. W., a 16 year old girl, was admitted to the University Hospitals Sept. 11, 1944. She had noted a progressive enlargement of her thyroid gland with increasing nervousness for two years. Later she experienced an increase of appetite but began to lose weight and noted that her heart beat rapidly. Her mother had observed prominence of her eyes for four months. No medication had been taken. On admission she had marked nervousness with tremor and definite exophthalmos. The thyroid gland was diffusely enlarged, and a loud bruit was heard over each lobe. The heart was not enlarged; the cardiac rhythm was regular and the rate 140; the blood pressure was 150 systolic and 68 diastolic. The basal metabolic rate was plus 53 per cent. Strong solution of iodine U. S. P., 10 minimis (0.61 cc.) three times a day, was given for four weeks. At the end of this period her pulse rate was 88, the blood pressure 128 systolic and 68 diastolic, and the basal metabolic rate plus 6 per cent. She had gained 3 pounds (about 1 Kg.). On October 13, after right subtotal lobectomy had been performed by Dr. Charles M. O'Leary, her pulse rate and blood pressure rose to such alarming heights that removal of the left lobe was not attempted. A stormy postoperative period ensued with evidence of thyroid crisis and bronchopneumonia. On the third post-

operative day administration of thiouracil was begun, 0.6 Gm. daily in divided doses. The administration of strong solution of iodine, 30 minims (1.85 cc.) daily, was continued. At the end of three weeks she had gained 10 pounds (4.5 Kg.), the pulse rate was 74, and the basal metabolic rate was plus 1 per cent. Subtotal lobectomy was performed on the left side, November 8, by Dr. Charles M. O'Leary.

*Description of Specimen.*—The specimen obtained at the first operation (Oct. 13, 1944) consisted of two pieces of thyroid tissue measuring 6 by 4 by 3 and 2 by 1 by 1.5 cm. The external surfaces were coarsely nodular. The nodules varied from 0.5 to 1 cm. in diameter. The cut surfaces had a meaty appearance with a



Figs. 5 (case 4), 6 and 7 (case 5).—Microscopic appearance of the thyroid glands of patients treated with strong solution of iodine U. S. P. followed by thiouracil. Figure 5 shows the appearance of the thyroid gland of a 31 year old woman (case 4) at the time of her second operation, following the administration of thiouracil. Figures 6 and 7 show the appearance of the thyroid gland of a 16 year old girl (case 5) after the administration of strong solution of iodine U. S. P. followed by thiouracil.  $\times 100$ .

colloid sheen; some of the vesicles were visible and measured up to 0.2 cm. in diameter. On microscopic examination (fig. 6) the acini varied in size and shape. They were lined by low columnar or cuboidal cells, had delicate or incomplete septums and were filled with light-stained or dark-stained colloid. Others contained large mononuclear cells with lightly stained cytoplasm and eccentrically placed nuclei. Sheets of interacinous cells were seen in places. Many of the acini had spurlike infoldings. In the connective tissue stroma in places there were aggregations of lymphocytes amounting to lymph follicle formation.

The specimen obtained at the second operation (Nov. 8, 1944) consisted of one lobe of thyroid gland with the isthmus, 6.5 by 4 by 3 cm. The external surface was coarsely lobulated. The cut surfaces had a meaty appearance, with a colloid sheen. On microscopic examination (fig. 7) there was marked variation in the size of the acini in the individual lobules. Some of the acini had frequent infoldings and were lined by tall columnar cells with the nuclei heaped up into several rows. Others were lined by cuboidal or columnar cells, were empty or contained some pink-stained amorphous material. Sheets of interacinous cells were seen in places. Occasional large acini were lined by flat cells and were filled with pink-stained colloid. Aggregations of lymphocytes amounting to lymph follicle formation, with overgrowth of germinal centers, were seen in the septums.

#### PATIENTS TREATED WITH THIOURACIL FOLLOWED BY STRONG SOLUTION OF IODINE U. S. P.

CASE 6.—B. H., a 33 year old housewife was admitted to the University Hospitals March 11, 1944. She had had signs and symptoms of exophthalmic goiter, developing over a period of eighteen months. She had been given strong solution of iodine U. S. P. for two months, this treatment being terminated four months prior to her admission. On admission, her thyroid gland was diffusely enlarged, and she had extreme nervousness, tremor and marked exophthalmos. Her heart was enlarged; the pulse rate was 120, and the blood pressure was 140 systolic and 70 diastolic. She weighed 96 pounds (43.5 Kg.), and the basal metabolic rate was plus 65 per cent. Thiouracil was administered, 0.6 Gm. daily in divided doses, for six weeks, at which time (April 22) the pulse rate was 92, the blood pressure 110 systolic and 65 diastolic, and the basal metabolic rate plus 49 per cent. She had gained 10 pounds (4.5 Kg.). At operation on April 24 (by Dr. L. J. Starry) the pulse rate rose to 140 and the blood pressure to 180 systolic and 100 diastolic. Only the right lobe of the thyroid gland was removed. The use of thiouracil, 0.6 Gm. daily, was continued, together with that of strong solution of iodine, 15 minimis (0.92 cc.) three times daily. On May 22 she weighed 117 pounds (53 Kg.), the pulse rate was 88, the blood pressure was 115 systolic and 80 diastolic, and the basal metabolic rate was plus 18 per cent. On June 5 left hemithyroidectomy was performed by Dr. C. E. Clymer.

*Description of Specimen.*—The specimen obtained at the first operation (April 24) consisted of a lobe of thyroid gland measuring 6 by 3.5 by 2 cm. The surface was coarsely lobulated; half of it was raw and the other half, covered by a thin membrane. The cut surfaces were pale pink and had a colloid sheen. On microscopic examination (fig. 8) there was marked variation in the size of the lobules and of the acini within the lobules. The acini were lined by cuboidal or columnar cells, with occasional spurs forming incomplete septums. The acini contained pink-stained colloid. Groups of interacinous cells were seen in places. Other acini were lined by low cuboidal cells. In the septums there were, here and there, aggregations of lymphocytes amounting to lymph follicle formation.

The specimen obtained at the second operation (June 5) consisted of a lobe of the thyroid gland measuring 5.5 by 4.5 by 2.5 cm. One surface was raw; the other was covered by a delicate membrane and was slightly lobulated. On the cut surfaces there were gray-pink fields having a colloid sheen and separated by delicate pale gray septums. On microscopic examination (fig. 9) various-sized acini lined by low cuboidal cells filled with pale pink-stained colloid were seen close to one another, separated by delicate or incomplete septums. An occasional



Figs. 8 and 9 (case 6) and 10 (case 7).—Microscopic appearance of the thyroid glands of patients treated with thiouracil followed by strong solution of iodine U. S. P. Figure 8 shows the appearance of the thyroid gland of a 35 year old woman (case 6) operated on while her basal metabolic rate was plus 49 per cent after she had received thiouracil for six weeks. Figure 9 shows the appearance of the specimen obtained at the second operation on this woman, following the administration of strong solution of iodine U. S. P. and thiouracil for six weeks. Figure 10 shows the appearance of the thyroid gland of a 37 year old woman (case 7) treated with thiouracil followed by strong solution of iodine U. S. P.  $\times 100$ .

lumen-had infoldings lined by tall columnar cells. The variation in the size of the acini was more marked in some fields than in others. Here and there groups of interacinous cells were seen. In focal areas there were some aggregations of lymphocytes amounting to lymph follicle formation.

CASE 7.—R. N., a 37 year old woman, was admitted to the University Hospitals Oct. 10, 1944. Three months prior to admission she began to suffer from nervousness, restlessness, fatigability and a rapid heart beat. Although her appetite increased, she gradually lost weight and noticed intolerance of heat. One month prior to admission she noticed enlargement of her neck. She had received no medication. On admission, nervousness and a fine tremor were apparent. There was widening of the palpebral fissures with a stare but no exophthalmos. The thyroid gland was moderately and diffusely enlarged. The blood pressure was 130 systolic and 84 diastolic; the pulse rate was 134 and the basal metabolic rate plus 53. Thiouracil was administered, 0.6 Gm. daily, and after one week the basal metabolic rate was plus 35 per cent. She continued to take thiouracil for the next three weeks as an outpatient. On November 7, four weeks after the beginning of the thiouracil treatment, she was readmitted. At this time the basal metabolic rate was plus 20 per cent, the pulse rate 88 and the blood pressure 140 systolic and 80 diastolic. She had gained 2 pounds (about 1 Kg.) and showed marked improvement in subjective symptoms. Strong solution of iodine U. S. P. was administered, 5 minijps (0.31 cc.) three times a day, in addition to thiouracil, 0.4 Gm. daily. After six days the basal metabolic rate was plus 6 per cent. On November 17 bilateral subtotal thyroidectomy was performed by Dr. John W. Cavanaugh.

*Description of Specimen.*—The specimen obtained consisted of both lobes of the thyroid gland with the pyramidal lobe, together weighing 35 Gm. The left lobe was 5 by 3 by 2 cm., the right 6 by 3 by 2 and the pyramidal lobe 2 by 0.6 cm. The external surfaces were coarsely lobulated. The cut surfaces had a colloid sheen and were even. On microscopic examination (fig. 10) there was marked variation in the size of the acini within the individual lobules and also in the size of the lobules. Most of the acini contained a faintly stained colloid or no colloid and were lined by flat cuboidal or columnar cells. The nuclei of the latter in places were heaped up into several rows. Other acini contained pale lavender-stained granular material. In some of the lobules the septums were delicate and incomplete and the covering cells almost flat. Groups of interacinous cells were seen in places. In focal areas there were aggregations of lymphocytes, plasma cells and large mononuclear cells.

#### COMMENT

All 7 patients when first seen had marked signs and symptoms of hyperthyroidism or exophthalmic goiter. On all, bilateral subtotal thyroidectomy was performed in one or two stages. Two patients had received preoperative therapy consisting of administration of thiouracil only; 3 were treated with strong solution of iodine U. S. P. followed by thiouracil, and 2 were treated with thiouracil followed by strong solution of iodine. In most instances, the intensity of the clinical signs and symptoms was lowered, the pulse and basal metabolic rates were near normal levels and the patients were gaining weight at the time of operation.

The morphologic observations were made on routine preparations stained with hematoxylin and eosin. Sections stained with Haidenhain's azocarmine and by Van Gieson's method were also studied.<sup>3</sup>

In the glands removed following the administration of thiouracil only, the acinous content stained lightly and was decreased or absent, and the cells lining the acini were low or tall columnar. These changes were quite similar to those seen in thyroid glands of untreated patients in an active state of hyperthyroidism.

More illuminating information was obtained from the study of the thyroid glands of the patients treated with strong solution of iodine U. S. P. followed by thiouracil. In these patients subtotal thyroidectomy was performed in two stages. In preparation for the first stage strong solution of iodine U. S. P. only was given. In preparation for the second stage thiouracil was given. Thus the effects of iodine and thiouracil could be compared and contrasted in the same patient. Following the administration of thiouracil, the cells of the acini changed from cuboidal to columnar, and the colloid disappeared or lessened in density and became vacuolated and scalloped.

In the glands removed after the administration of thiouracil followed by strong solution of iodine U. S. P., a refilling of the acini with colloid could be observed, together with a change of the lining cells from columnar to low columnar or cuboidal.

According to these observations, the acinous colloid diminishes in quantity and density or disappears under the influence of thiouracil. The variance between structure and function of the thyroid gland following the administration of thiouracil supports the assertion that thiouracil inhibits the production of new colloid and does not interfere with the use of the available colloid.

The phenomena involved may be understood more easily if one assumes that even under ordinary conditions the acini of the thyroid gland are in various phases of function. These might be called resorptive phase, secretory phase and resting phase.

In the resorptive phase the majority of the acini in each lobule are lined with columnar or low columnar cells and contain light-stained, vacuolated and scalloped colloid. Such is the microscopic appearance of the thyroid gland in untreated patients with hyperthyroidism and in patients treated with thiouracil only.

In the secretory phase the majority of the acini in each lobule are lined with low columnar or cuboidal cells and contain deeper or darker stained homogeneous colloid. Such is the microscopic appearance of the acini of the normal thyroid gland and of the glands of patients with hyperthyroidism treated with strong solution of iodine

3. Halpert, B., and Thuringer, J. M.: Anat. Rec. 91:16, 1945.

U. S. P. These changes are also seen in the thyroid glands of patients with hyperthyroidism treated with thiouracil followed by strong solution of iodine.

In the resting phase the majority of the acini in each lobule are rather large, are lined with low cuboidal or flat cells and contain deeply or darkly stained homogeneous colloid. This is the microscopic appearance of the acini of thyroid glands with colloid hyperplasia and of the glands of patients with hyperthyroidism treated with strong solution of iodine U. S. P. for a prolonged period.

Thus the effect of thiouracil may be described as intensifying the resorptive phase, and that of strong solution of iodine U. S. P., the secretory phase. Thiouracil and strong solution of iodine U. S. P. administered alternately produce a balance of the functional phases.

#### SUMMARY

According to these observations, the acinous colloid diminishes in quantity and density or disappears under the influence of thiouracil. The variance between structure and function of the thyroid gland following the administration of thiouracil supports the assertion that thiouracil inhibits the production of new colloid and does not interfere with the use of the available colloid.

# SCLEREMA ADIPOSUM NEONATORUM OF BOTH INTERNAL AND EXTERNAL ADIPOSE TISSUE

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AND  
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HARDENING of adipose tissue has been described in medical literature under a wide variety of names and has been attributed to many different causes. When pathologic sections have been taken they have revealed strikingly similar lesions in many of the reported cases even though there has been wide variation in the clinical manifestations. The lesion consists of varying degrees of degeneration, necrosis and sometimes crystallization of adipose tissue together with chronic inflammation more or less granulomatous in nature, often with foreign body giant cells and with a tendency to heal by formation of scar tissue. Most of the reports on this subject make no mention of the state of the perivisceral adipose tissue, and there is often no indication that this tissue was examined microscopically. The pathologic studies of many reported cases have been limited to biopsy material. This limitation and the fact that the cutaneous manifestations of the condition are so striking both clinically and pathologically have led to the generally accepted opinion that such conditions as subcutaneous fat necrosis of the newborn and nonsuppurative panniculitis are confined to the external adipose tissues. This supposition has even been used as a premise by those who assert that the former is caused by obstetric trauma. The case to be described presented widespread involvement of adipose tissue, both internal and external, with lesions which were histologically typical of subcutaneous fat necrosis of the newborn. No other such case was found recorded in the available literature.

## REPORT OF A CASE

A Negro infant was born in the Cincinnati General Hospital on Jan. 19, 1943. His 34 year old mother had had three previous pregnancies. These had resulted in the delivery of a full term, 9½ pound (4,309 Gm.) child, now 16 years old, living and well, and two abortions, ten and twelve years later, respectively. A few months before the second abortion she was hospitalized because of pelvic inflammatory disease. One year before the present patient was born, she was found to have diabetes mellitus. She was given insulin and a diet

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which restricted her consumption of butter, milk and fruit. During this fourth pregnancy she had vomited frequently and had lost weight. Nine days before delivery she was admitted to the hospital because of a cyst of one of Skene's glands with urethral stricture. She had not received insulin for two weeks. During fasting the blood sugar was 142 mg. per hundred cubic centimeters. After the diabetes had been brought under control with diet and regular dosage of insulin, labor was induced by rupturing the membranes and releasing about 1,000 cc. of amniotic fluid. Birth occurred twenty-six hours and fifty minutes later. The infant weighed 6 pounds 13½ ounces (3,104 Gm.). He was cyanotic and had paralysis of both arms, that of the left arm being the more marked. He was given 5 per cent dextrose in isotonic solution of sodium chloride subcutaneously and 3.2 mg. of a synthetic preparation of vitamin K (Synkayvite). Oxygen was used to relieve the cyanosis. The next day a pediatric consultant noted petechiae on both cheeks and edema of the skin over the legs and the trunk. On the third day after birth the baby became cyanotic after feeding, and his respirations became irregular. He was transferred to the pediatric service, where he seemed to improve somewhat under routine care during the next few days.

Examined on the ninth day of life, he appeared to be well developed and well nourished. His temperature was 98 F., and his respiratory rate was 20. Hard, nonpitting edema of the subcutaneous tissues was noted in the scalp, the face, the body and the extremities, excluding only the palms, the soles and the genitalia. The facial edema made sucking difficult. There was an ulcer, 2 cm. in diameter, in the scalp over the right occipitoparietal region. There was ulnar deviation of both wrists, and the baby kept his arms flexed at the elbows. Reflexes were absent in the upper extremities and hypoactive in the lower ones, and there was a positive Babinski sign. The heart, the lungs and the abdomen were essentially normal.

On Jan. 21, 1943 the following data were recorded: hemoglobin content, 22 Gm.; red blood cell count, 6,200,000; white cell count, 34,000; differential count, polymorphonuclears 70 per cent, lymphocytes 19 per cent, eosinophils 1 per cent, normoblasts 57 per cent. On this date the blood sugar value was 51 mg. per hundred cubic centimeters. On January 27 the blood sugar values were as follows: one-half hour specimen, 101 mg.; one and one-half hour specimen, 99 mg.; three and one-half hour specimen, 63 mg. The mother's red blood cells were shown to contain an Rh factor. On January 10 a Kahn test of the mother's blood was negative. On February 1 a Kahn test of the baby's blood was negative; the icteric index was 30; the serum protein amounted to 4.38 Gm. per hundred cubic centimeters.

When the blood sugar was found to be low, the baby was given 60 cc. of 5 per cent dextrose in isotonic solution of sodium chloride subcutaneously. For his feeding an evaporated milk formula was adopted (1:2 mixture, 3 ounces [88.5 cc.] in six feedings). The baby seemed to improve until the fifth day of life when his respirations again became irregular. He was placed in a Hess bed with oxygen being given as needed. His temperature was around 97 F. most of the time. On the fourteenth day of life a brawny, red, firm, nonpitting induration developed over the back from the waist to the shoulders and extended to the clavicular areas. At the same time the baby was found to have a bilateral bloody nasal discharge. The temperature rose to 101.8 F., the stools became liquid, and the urine was bile stained. The icteric index at this time was 30. The baby was given water by gavage. On the sixteenth day of life he was found dead in his crib.

At autopsy, about twenty-four hours after death, the body weighed 2,975 Gm. and was 48 cm. in length. There was brawny thickening of the skin of the back.

The skin over the rest of the body and the extremities felt firm, as though it were frozen. There was a small ulcer in the right occipitoparietal portion of the scalp. The scleras were slightly icteric. The foreskin of the penis was adherent. There was an abundance of firm adipose tissue beneath the thin, stretched skin. The panniculus varied in color and consistency, but none of it appeared normal. It was all light yellow or white and pasty, in some areas appearing like tallow, and in other places being crumbly and chalky. In some regions, particularly in the left axilla and the left clavicular region and in the perithymic adipose tissue, the fat was streaked with deep pink, watery tissue which appeared to be necrotic. Lymph nodes draining these regions were cherry red, swollen and softer than normal. The subcutaneous tissue of the back was much firmer than normal and seemed to be streaked with fibrous tissue. The right brachial plexus appeared white and glistening, but the left one was dull, pinkish gray and embedded in pink edematous adipose tissue. There was no frank hemorrhage but much congestion in this region. No rupture or other evidence of trauma of the brachial plexus was found, but it was difficult to separate the nerves from the surrounding fat because of interlacing strands of scar tissue. The thymus was swollen, soft and congested. Small subendocardial hemorrhages were scattered over the papillary musculature of the right ventricle. The lungs were subcrepitant and had a few scattered petechial hemorrhages beneath the pleura. The spleen was about one and one-half times the normal size, because of acute congestion. The liver was swollen and slightly icteric. The gastroenteric and genitourinary tracts appeared normal.

Microscopic sections revealed foci of necrosis and beginning acute inflammation, associated with petechial hemorrhages and colonies of short-chained cocci, in various organs and tissues widely disseminated throughout the body. The oldest lesion of this type was the ulcer of the scalp, which appeared to be less than a week old. The baby obviously had terminal septicemia.

The most remarkable type of lesion was found widely disseminated in the adipose tissues of the body. Lesions of this type were frequently found unassociated with the acute septicemic lesions just described, and were definitely much older than any of them. They were characterized by extensive degeneration and necrosis of adipose tissues, with the fats precipitated as needle-shaped crystals, some of which were doubly refractile under the polarizer. The cytoplasm of many of the fat cells stained faintly with hematoxylin and eosin, which is unusual in paraffin sections. Associated with this morphologic evidence of changes in the fat of the adipose tissues was a marked chronic inflammatory reaction with fibrous tissue proliferation and the presence of numerous macrophages, foreign body giant cells and other inflammatory cells. In some regions there was extensive proliferation of fibrous tissue in and around the fat lobules, some of which was hyalinized and appeared to be more than two weeks old. This indicates that the lesion began before the child was born.

The ulcer in the scalp was superimposed on an area of fat necrosis and inflammation. The left brachial plexus was embedded in a similar lesion (fig. 1). Several of the large nerves were surrounded by inflammatory exudate and scar tissue. Lesions in the adipose tissue surrounding the right brachial plexus were of less severity than those on the left side.

At autopsy the only internal adipose tissue which appeared to be abnormal was that around the thymus; this fat seemed to be pinker and firmer than normal. For this reason sections were taken from it for microscopic study. At that time no attempt was made to include fat with the sections of other viscera. On examination of the microscopic sections, the fat of the perithymic region presented not only

acute septicemic lesions but also the typical granulomatous and necrotizing chronic lesions of sclerema adiposum. A careful search of the other routine sections revealed similar lesions in bits of adipose tissue included in one section each of the

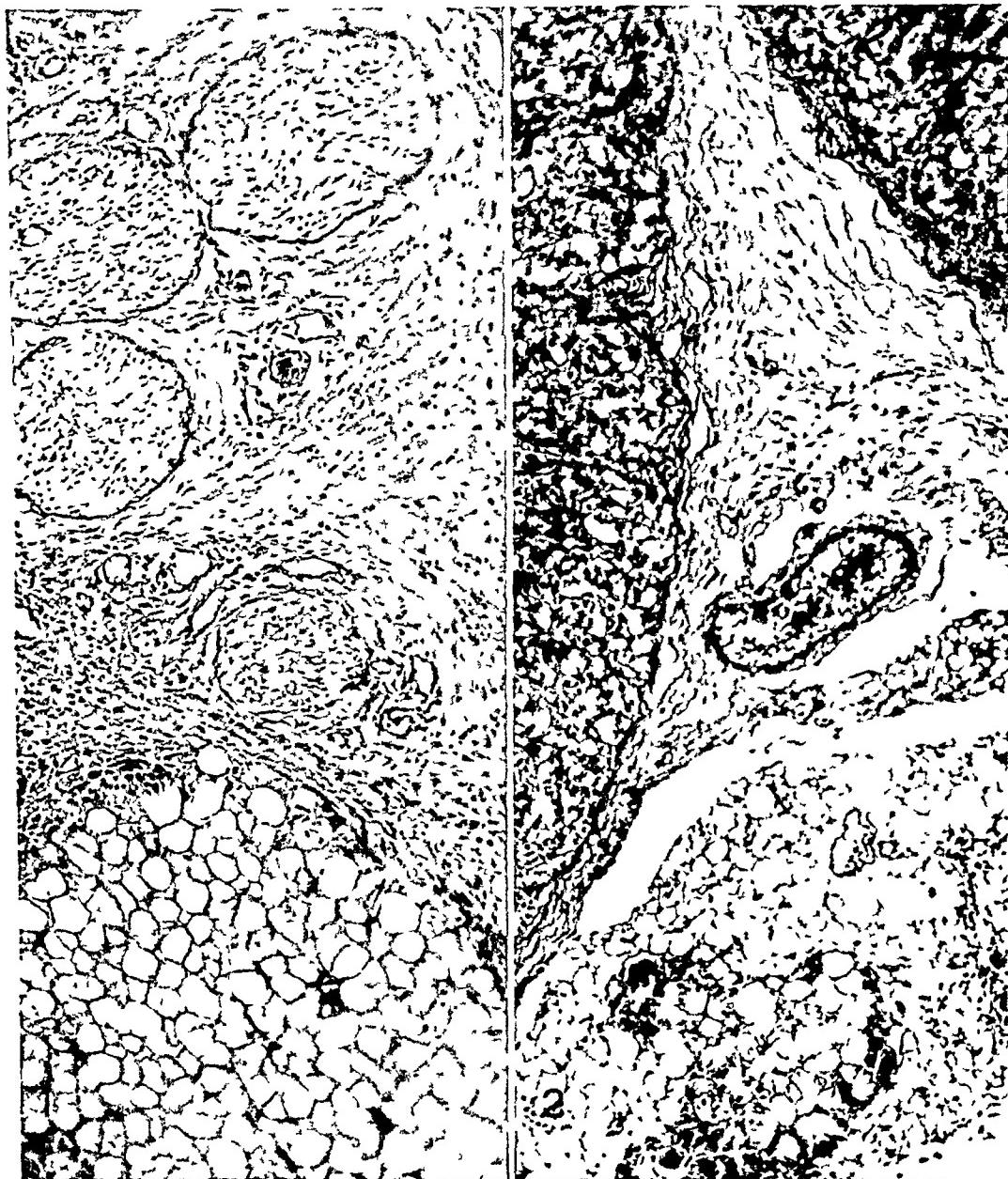


Fig. 1.—Sclerema adiposum around the nerves of the brachial plexus in the left axilla. Hematoxylin and eosin;  $\times 110$ .

Fig. 2.—Sclerema adiposum in the periadrenal fat. Note the giant cells in the outer margins of the nodules. Hematoxylin and eosin;  $\times 110$ .

pancreas and the kidney. Fortunately, the infant's body was still in the ice box, since it was one for city disposal. It was still in good condition, although ten days had elapsed since death. Additional samples were taken from adipose tissue in

a wide variety of locations. Microscopic sections of samples of adipose tissue from every location except the scrotum revealed lesions similar in every respect to those found in the panniculus. They included sections from numerous sites near the various viscera, in the mesentery, at the root of the diaphragm and in peribronchial and periaortic regions. No such lesions were found anywhere except in the adipose tissues. The pathologic diagnosis was as follows: terminal septicemia; sclerema

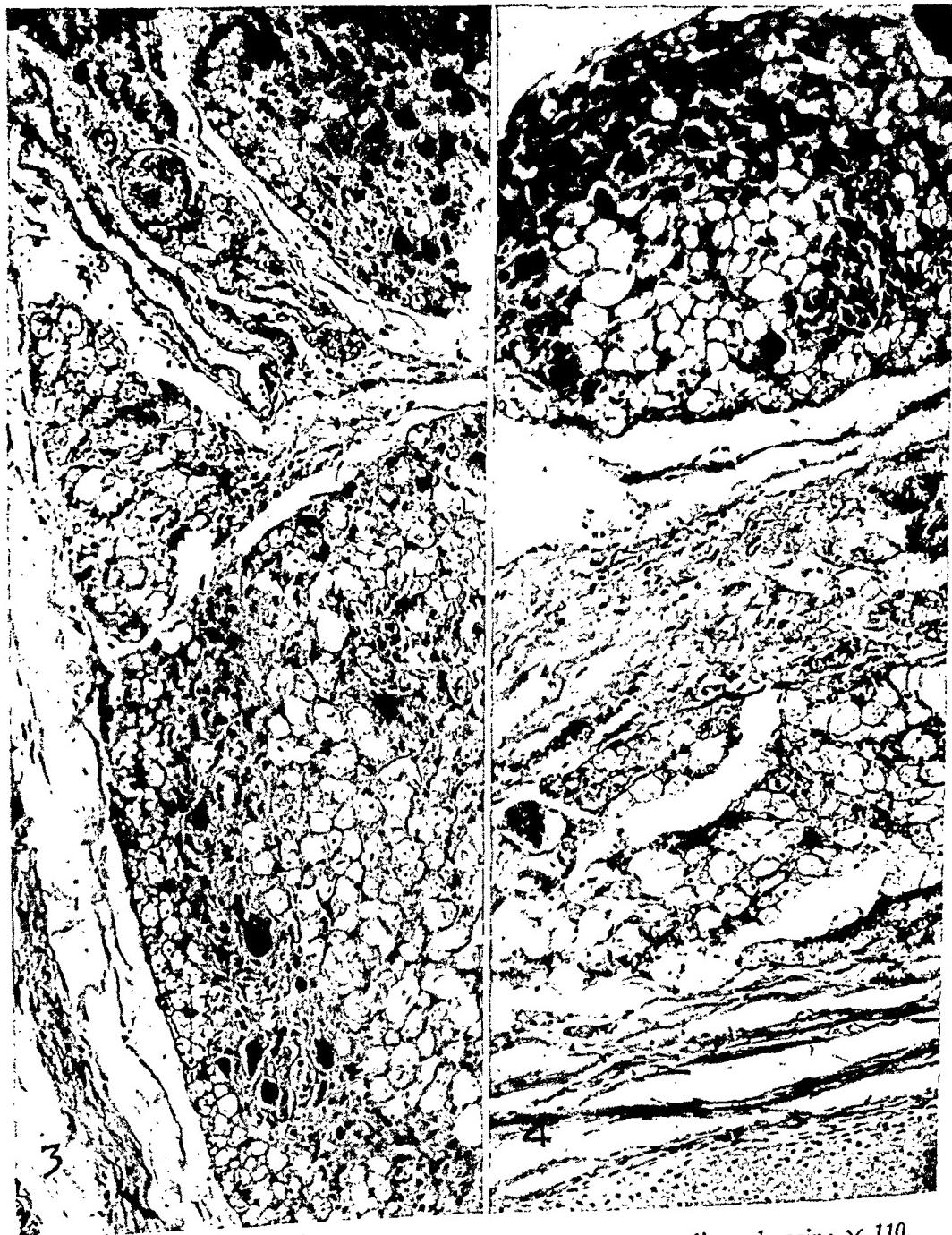


Fig. 3.—Sclerema adiposum in the perithymic fat. Hematoxylin and eosin;  $\times 110$ .  
Fig. 4.—Sclerema adiposum in the peritracheal fat. Hematoxylin and eosin;  $\times 110$ .

adiposum neonatorum with widespread involvement of subcutaneous and perivisceral adipose tissues; scarring of axillary fat around the left brachial plexus; acute ulceration of a sclerotic region in the scalp.

#### COMMENT

It cannot be stated at present whether this case represents an exception to a general rule concerning the distribution of such lesions of adipose tissues, or whether the internal lesions have been missed heretofore as they were nearly missed in this case. Blocks of infant's viscera taken routinely for microscopic study usually include little adipose tissue. In the cases of adults, pathologic studies of hardening of adipose tissue, such as occurs in febrile nodular nonsuppurative panniculitis and in traumatic fat necrosis of the female breast, have been limited usually to biopsy material. In some of these cases, particularly those of fat necrosis associated with localized trauma caused either by mechanical or by chemical agents, it seems reasonable to suppose that the lesion would remain localized. But in other cases in which there has long been known to be widespread involvement of subcutaneous fat, as in subcutaneous fat necrosis of the newborn and in nonsuppurative panniculitis, such an assumption is unjustifiable until careful search has been made of microscopic sections of the internal adipose tissues in more than just a few cases.

A review of the available literature on nonsuppurative panniculitis revealed only 2 cases in which an autopsy was done during the active stage of this condition. In 1943 Miller and Kritzler<sup>1</sup> reported such a case, in which the examination was limited to "the study of one cutaneous lesion and the organs of the abdomen and chest." The authors found no lesion such as those in the panniculus in the perirenal, mesenteric or pericardial fat, but they saw occasional "lymphocytes, and phagocytes in the epicardial fat." They did not consider the presence of these cells significant. In 1944 Spain and Foley<sup>2</sup> performed autopsy in a case of nonsuppurative panniculitis, in which they found nodules in the mesenteric, omental and pretracheal fat grossly similar to those in the panniculus. However, they thought these lesions were microscopically "dissimilar to the pannicular lesions" and grouped them under "pancreatic fat necrosis" in their diagnosis.

The factors most frequently named as possibly playing a role in the genesis of subcutaneous fat necrosis of the newborn have been reviewed by Fox,<sup>3</sup> Gray<sup>4</sup> and many others. Some of the factors named are as follows:

1. Miller, J. L., and Kritzler, R. A.: Arch. Dermat. & Syph. **47**:82, 1943.
2. Spain, D. M., and Foley, J. M.: Am. J. Path. **20**:783, 1944.
3. Fox, H.: Arch. Dermat. & Syph. **27**:237, 1933.
4. Gray, A. M. H.: Brit. J. Dermat. **45**:498, 1933.

1. Obstetric trauma
2. Low temperature of the body, causing fat to solidify and act as a foreign body
3. Deficiency of olein in the adipose tissue, causing fat to solidify at a higher temperature than is usual for normal fat, possibly at normal body temperature
4. Presence of an abnormal lipolytic ferment
5. Glandular dyscrasia resembling myxedema
6. Varying combinations of the aforesaid factors.

In the present case, although there was a long, somewhat difficult labor, obstetric trauma cannot possibly account for all of the lesions, especially those in well protected internal fat depots. Also, the scar tissue present in some of the fat lobules, particularly that in the region of the left brachial plexus, appeared to be older than the age of the infant. Also, the baby was born with paralysis of the brachial plexus, the only cause found being constriction due to inflammatory reaction and fibrosis associated with the lesions of sclerema adiposum in the surrounding axillary adipose tissue. This baby's temperature ranged around 97 F. most of the time until the onset of the terminal infection; therefore low temperature may have been a factor in accelerating the process after birth.

The most plausible explanation of the lesions in this infant seems to be that they were caused by some generalized disturbance of fat metabolism, the origin of which antedated the birth of the child: The presence of diabetes mellitus and possibly some nutritional deficiency in the mother furnishes interesting ideas, though only for conjecture at present. A review of the reported cases in the literature furnishes proof that not all mothers of such infants have been diabetic.

Chemical studies have been made on subcutaneous tissues by Langer,<sup>5</sup> Smith,<sup>6</sup> Harrison,<sup>7</sup> Chen<sup>8</sup> and others. They furnish substantial evidence that the subcutaneous fat of infants contains a smaller proportion of olein than does that of adults, but opinions vary as to whether there is any essential chemical difference between the fat of normal and that of scleremic infants.

The nomenclature which has been applied to the type of lesion under discussion is in a state of confusion. Most of the names used for this condition have been based on the clinical manifestations of the lesion, and these have presented wide variations. Bailey<sup>9</sup> in reporting 5 cases of

5. Langer, L.: Wien. med. Presse **22**:1375 and 1412, 1881.

6. Smith, C. S.: J. Cutan. Dis. **36**:436, 1918.

7. Harrison, G. A.: Arch. Dis. Childhood **1**:63 and 123, 1926.

8. Chen, T. T.: Nat. M. J. China **16**:360, 1930.

9. Bailey, R. J.: J. A. M. A. **109**:1419, 1937.

nonsuppurative panniculitis lists the following clinical conditions which presumably result from mechanical and chemical damage to adipose tissue and which present similar lesions microscopically: (1) subcutaneous fat necrosis of the newborn, (2) traumatic fat necrosis of the breast, (3) atrophy of fat induced by injection of insulin, (4) paraffinoma, (5) ischemic fat necrosis, (6) lipogranulomatosis, (7) oleogranuloma.

To be added to this list are the numerous terms which Gray<sup>4</sup> found in the literature, applied to the first condition named. After reviewing the long and devious history of the nomenclature of that condition he became a champion of the term "sclerema neonatorum." But this term, which literally means "hardening of the newborn," has been applied to "hardening" conditions in which no change was found in the adipose tissues.

The word "sclerema" comes from the Greek word *σκληρός* which means "hard." Therefore, "sclerema adiposum" seemed to us to be a good generic term to apply to the lesion in the present case. This term could be applied advantageously to the lesion in all of the conditions just listed, since at some stage of each of them there is hardening of the adipose tissues. Modifying terms could be added to this root term to define further the condition present in a given patient. Examples: "sclerema adiposum neonatorum;" "nodular nonsuppurative sclerema adiposum"; "traumatic sclerema adiposum of the breast." By thus emphasizing the factor common to all of these conditions one might hope to determine sooner their etiology and genesis. Also, the use of this term would help to differentiate those cases in which "hardening" involves adipose tissues from those in which no such lesions are found.

The factor or factors which initiate this lesion are unknown at present. The morphologic aspect of the lesion suggests some disturbance of the nutrition or of the metabolism of fat cells, which so changes the nature of the fat that it acts as a foreign body and thus leads to the characteristic inflammatory reaction and degenerative changes.

Adipose tissue is indeed, as Wells<sup>10</sup> has emphasized, a neglected subject. No longer should it be considered as just so much connective tissue loaded with stored simple fats. Evidence is accumulating to support the concept that the adipose tissue of the body is structurally, developmentally and functionally a distinctive special tissue, more after the order of the ductless glands. The pathologic study of this tissue should not be restricted to an occasional biopsy taken by the dermatologist but should include careful routine examination by the general pathologist.

10. Wells, H. G.: J. A. M. A. 114:2177 and 2284, 1940.

## SUMMARY

In a case of sclerema adiposum neonatorum the lesions were numerous and widely distributed in both the internal and the external adipose tissues of the body. There was evidence that the condition began before birth. The nomenclature of this and other clinical conditions which present similar lesions invites attention, and the suggestion is made that the words "sclerema adiposum," meaning "hardening of adipose tissue," be included in whatever diagnostic term is used pathologically for each of these conditions.

## "ALVEOLAR CELL TUMOR" OF THE LUNG

*Further Evidence of Its Bronchiolar Origin*

PETER A. HERBUT, M.D.

PHILADELPHIA

REPORTS of tumors purporting to arise in cells lining pulmonary alveoli continue to appear in medical literature. In a previous communication<sup>1</sup> an attempt was made to analyze available data and determine whether there is any convincing evidence that a primary tumor can arise in this location, or whether the arrangement of tumor cells along the alveolar septums is merely an adaptation of cancer cells arising from the bronchial or the bronchiolar epithelium. From a review of the literature, a study of various chronic inflammatory lesions of the lungs and an analysis of 90 cases of primary pulmonary carcinoma the following conclusions were reached: 1. There is no unanimity of opinion among histologists and embryologists as to whether pulmonary alveoli are lined by epithelial or by mesothelial cells, or indeed by any cells. 2. In most if not all instances the so-called regenerated alveolar epithelium seen in chronic inflammatory lesions of the lungs arises not from septal cells but from the basal cells of the bronchi and particularly from those of the bronchioles. 3. Primary carcinoma of the lung arises from the basal cells of bronchi and bronchioles, and further differentiation or lack of differentiation determines the distribution of these cells in relation to the rest of the pulmonary parenchyma. If they form cuboidal or columnar cells, these will line the alveolar septums in accordance with their inherent property to line spaces and so will produce the well known alveolar distribution. If the cancerous cells form anaplastic or squamous cells, these either will be irregularly distributed throughout the lungs or will occupy the alveolar spaces just as does the exudate in an ordinary type of pneumonia. It was further concluded that since primary bronchiogenic carcinoma is extremely pleiomorphic, it rarely is found to be of one uniform cell type and that in a single specimen there are often mixtures of cuboidal, columnar, anaplastic and squamous cells in alveolar, pneumonic or irregular distribution.

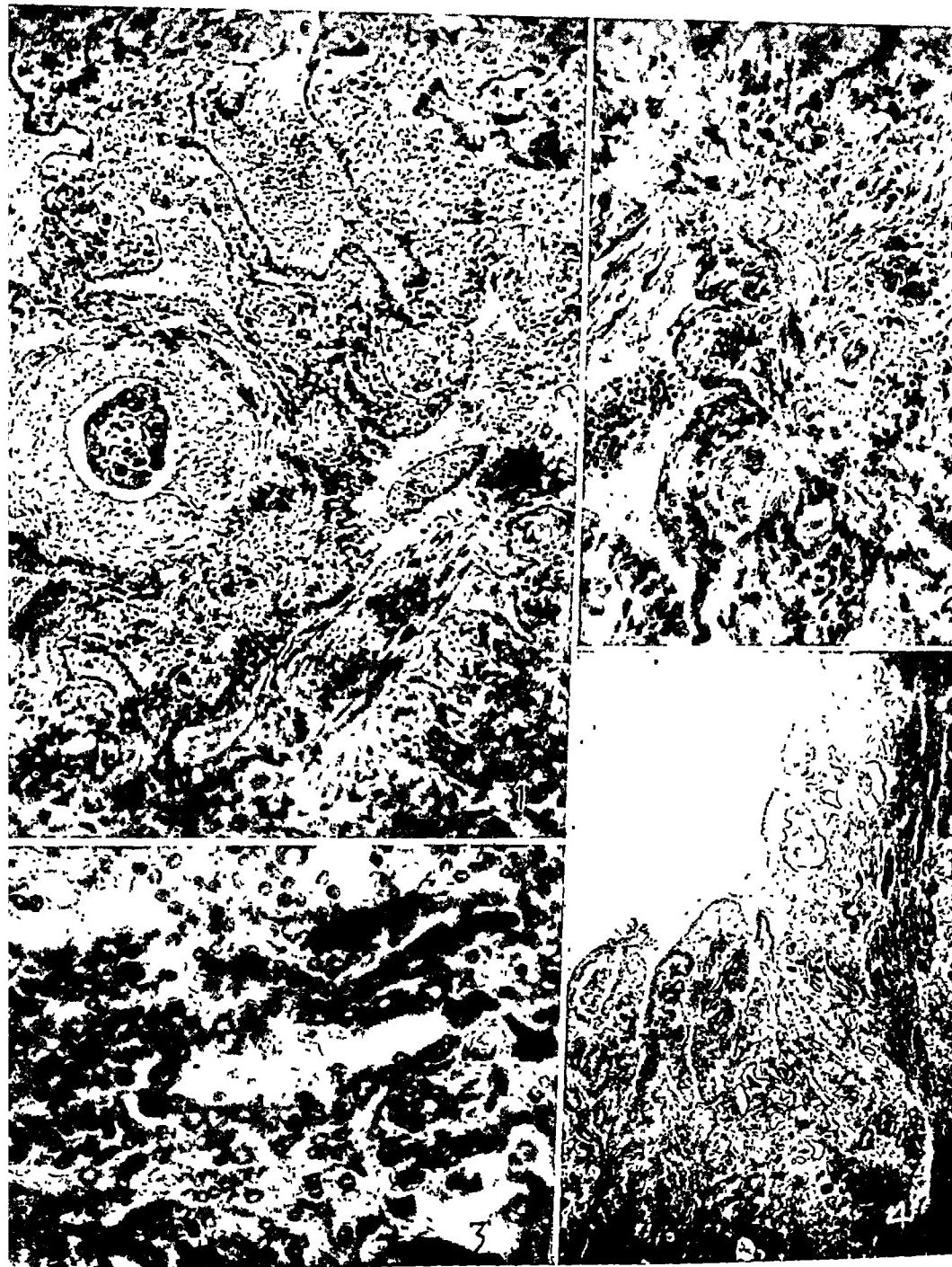
After the aforementioned article was accepted for publication, Taft and Nickerson<sup>2</sup> contributed a report of 2 cases of pulmonary mucous

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From the Clinical Laboratories, Jefferson Medical College Hospital.

1. Herbut, P. A.: Am. J. Path. 20:911, 1944.

2. Taft, E. B., and Nickerson, D. A.: Am. J. Path. 20:395, 1944.



(See legends on opposite page)

epithelial hyperplasia in which they expressed the belief that the hyperplasia originated in the alveolar lining cells but added that an "origin from goblet cells of bronchiolar mucosa cannot be excluded." Subsequently, Ikeda<sup>3</sup> recorded 2 typical cases of alveolar cell tumor and 2 others which were less typical but which nevertheless were designated as such; Wood and Pierson<sup>4</sup> reported 1 case, and Geever, Carter, Neubuerger and Schmidt<sup>5</sup> added 5 cases which had not been previously reported. The latter authors stated that the microscopic examination in all the cases which they presented "favored the belief that the origin of the neoplastic growth was in the alveolar walls. A completely satisfactory proof of this opinion cannot of course be offered."

The question whether primary carcinoma can or cannot arise in cells lining the alveoli is of more than academic interest, for those who contend that such an origin is possible also say that the tumors arise in many foci in both lungs. If this is so, the lesion will be forever hopeless from a therapeutic standpoint. If, on the other hand, the premise that the so-called alveolar cell carcinoma arises from a single focus in a small bronchus or a bronchiole is correct, the prognosis in the future may be even better than that of the more commonly recognized bronchiogenic carcinoma. The latter is frequently so close to the hilus of the lung that complete surgical extirpation is impossible. This is not true of tumors that originate in the more peripheral portions of the bronchial tree, and if a diagnosis is made early, complete removal should be possible. In respect to an early diagnosis some ray of hope is perhaps offered by the current practice of establishing cancer prevention clinics

3. Ikeda, K.: Am. J. Clin. Path. **15**:50, 1945.

4. Wood, D. A., and Pierson, P. H.: Am. Rev. Tuberc. **51**:205, 1945.

5. Geever, E. F.; Carter, H. R.; Neubuerger, K. T., and Schmidt, E. A.: Radiology **44**:319, 1945.

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#### EXPLANATION OF PLATE.

Fig. 1.—A bronchiole lined in part with a single layer of cuboidal cells and in part with a pseudostratified layer of epithelium is shown. The epithelium has extended through the bronchial wall to line adjoining alveolar septums. A blood vessel is filled with tumor cells. Hematoxylin and eosin;  $\times 100$ .

Fig. 2.—Higher magnification of a portion of the bronchiole illustrated in figure 1 at a different level, showing a lining of cuboidal and pseudostratified epithelium which in the lower portion is penetrating the wall. Note the strands of smooth muscle within the wall of the bronchiole. Hematoxylin and eosin;  $\times 200$ .

Fig. 3.—An alveolus lined with a single layer of cuboidal cells, three of which are in a state of mitosis. The alveolar septums are greatly broadened and disrupted by extravasated erythrocytes. Hematoxylin and eosin;  $\times 400$ .

Fig. 4.—Section of the gallbladder showing a zone of transition between the normal epithelium and the cancer cells that line villi and infiltrate the underlying structures in an adenomatous formation. Hematoxylin and eosin;  $\times 25$ .

where routine periodic roentgenograms of the chest and the finding of cancer cells in bronchial secretions<sup>6</sup> might disclose the lesion while it is still amenable to operation.

It is the purpose of this report to present (1) a very early "alveolar cell tumor" arising in a bronchiole, (2) a primary carcinoma of the gallbladder with metastases in the lungs that grossly and microscopically were indistinguishable from primary "alveolar cell tumor" of the lung, and (3) an analysis of the distribution of metastatic carcinoma of the lungs to see whether, according to cell type, the metastases follow the pattern of primary bronchiogenic neoplasms.

#### EARLY "ALVEOLAR CELL TUMOR" ARISING IN A BRONCHIOLE

A white woman 69 years old was admitted to the Jefferson Medical College Hospital with a cystocele, a rectocele and prolapse of the uterus. At operation the cystocele and the rectocele were repaired, the uterus was fixed to the anterior abdominal wall and the appendix was removed. Nine days later a sharp pain developed in the lower portion of the right half of the chest, from which she promptly recovered. On the thirteenth day she suddenly became extremely dyspneic and died in shock thirty minutes later.

At necropsy the operative sites were clean. Each internal iliac vein contained a thrombus lying free in the lumen. The main pulmonary artery and each of its branches were filled with a coiled gray embolus similar to the thrombi in the iliac veins. The lateral portion of the lower lobe of the right lung disclosed a deep red infarct 4 cm. in diameter. The lungs were congested and edematous. Nowhere were there tumor nodules. The mediastinal nodes were not enlarged and the remaining organs showed no contributory changes.

Microscopic sections from the site of the infarct of the lower lobe of the right lung disclosed a bronchiole lined in part with a single layer of cuboidal cells and from these a gradual piling up of cells to form a pseudostratified type of epithelium (figs. 1, 2 and 3). At several points the latter had broken through the bronchiolar wall and lined the surrounding alveolar septums with cuboidal or somewhat flattened cells one, two or three layers thick. Although most of the cells were regular, some showed considerable variation in shape and size, as well as hyperchromatism and mitosis. Two blood vessels on each side of the bronchiole were filled with tumor cells similar to those just referred to. Several bands of smooth muscle, which with Van Gieson's stain appeared yellow, were present within the wall of the bronchiole, and throughout the alveolar septums and spaces there was much erythrocytic extravasation. When this lesion was discovered, the right lung was reexamined. No grossly visible tumors were present. Many new sections were then made of the lung in the vicinity of the infarct and between the infarcted area and the hilus. None of the sections from the former site showed tumor, but in one of the sections from the latter site tumor cells were found within a vessel of a larger caliber than those noted in the first sections. Metastases were not found in the lungs or in any of the other organs, and there were no other contributory findings.

6. Herbut, P. A., and Clerf, L. H.: Bronchiogenic Carcinoma Diagnosed by Cytologic Study of Bronchoscopically Removed Secretions, J. A. M. A., to be published.

CARCINOMA OF THE GALLBLADDER SIMULATING PRIMARY  
"ALVEOLAR CELL TUMOR"

A white woman 59 years old was admitted to the hospital with a history of griping and colicky pain in the right upper quadrant of the abdomen and constipation of two months' duration. The pain was aggravated by eating, but there was no nausea, and vomiting occurred on only one occasion. Examination disclosed a hard mass 3 cm. in diameter in the region of the gallbladder and generalized tenderness in the right upper quadrant of the abdomen. There were no pulmonary signs or symptoms. Roentgenograms of the chest disclosed enlarged hilar shadows with streaks of density radiating into both lung fields. At operation an irremovable cancer of the gallbladder was found. Five days later she had some indrawing of the sternum and rales in the chest. These persisted, and she died dyspneic twelve days after the operation.

Necropsy disclosed slight enlargement of the gallbladder. At its fundus there was a firm, gray, flat tumor that measured 5 cm. across and 1.3 cm. in thickness. It was sharply demarcated from the surrounding mucosa and raised 3 mm. above its surface. Its inner surface was rough and superficially ulcerated, and it penetrated the entire wall to include the serosa. Since most of the tumor was at the tip of the gallbladder and along its inferior border, it did not penetrate the liver substance. The remaining gallbladder wall was several times the normal thickness, firm and indurated, but grossly showed no tumor. The lumen contained thick gray mucoid material and several polygonal black calculi measuring as much as 1.5 cm. in diameter. The cystic duct was stenosed by neoplastic tissue, but the hepatic ducts and the common bile duct were patent. The draining lymph nodes were replaced with mucoid tumor tissue. The liver weighed 1,550 Gm. and contained many circumscribed gray tumors measuring as much as 2 cm. in diameter. There were several cancer nodules in the serosa of the sigmoid colon and one in the left adrenal gland measuring 1.5 cm. in diameter. The peritoneal and each pleural cavity contained 1,500 cc. of straw-colored fluid. The pleural surfaces of each lung were dull but contained no tumors. The pulmonary parenchyma, however, was extensively infiltrated throughout by light gray cancer tissue. Many of the foci were sharply circumscribed and measured 2 to 3 mm. in diameter, while others were more confluent, less sharply defined, and measured 4 cm. across. The centers of many of the larger masses showed beginning necrosis and were filled with mucoid material. The trachea and bronchi were dissected carefully, and no primary tumors were found. The mediastinal lymph nodes were replaced with a gray mucoid cancer tissue, and there were tumor nodules in the diaphragm. The remaining organs showed nothing relevant.

Microscopic sections made of the gallbladder wall at the junction of the tumor with the mucosa showed a rapid transition between the latter and the cancer tissue (fig. 4). The cells became slightly taller and more irregular. Often they were still cuboidal and both covered the gallbladder villi and lined underlying spaces in a single cell layer. Their cytoplasm was pink and abundant and occasionally contained vacuoles. The nuclei were hyperchromatic and varied considerably in size but were usually basilar in position. In some areas the cells were columnar, one to three layers thick and, as before, formed regular acini, while in other areas cuboidal, columnar or irregular cells were loosely arranged, showing no glandular formation. In a few of the sections the tumor was practically confined to the mucosa and the immediately subjacent connective tissue, although in most sections it involved the muscle coats and the serosa. In the

latter there was a marked desmoplastic reaction about the tumor cells. Many sections of the lungs were examined, and all showed cancer cells lining the alveolar septums (fig. 5). The cells were cuboidal or columnar and one or several layers deep. The nuclei were basilar; sometimes they were quite regular, but at other times they varied in shape and size and exhibited considerable hyperchromatism. Mitoses were not seen. Occasionally several nuclei were present within a single cell. The cytoplasm was abundant, pink and at times vacuolated. Papillae projecting into the lumens were abundant. Usually the cells were closely adherent to the septum, but sometimes they were separated from it. In addition to the peripheral rim of cells, the alveoli occasionally contained tumor in solid masses or in adenomatous formation. In spotty areas they were filled with mucoid material, which was seen to arise from the surrounding epithelial cells. A desmoplastic reaction was rarely seen, but when it was present the entire parenchyma of the lung in these small foci was replaced with

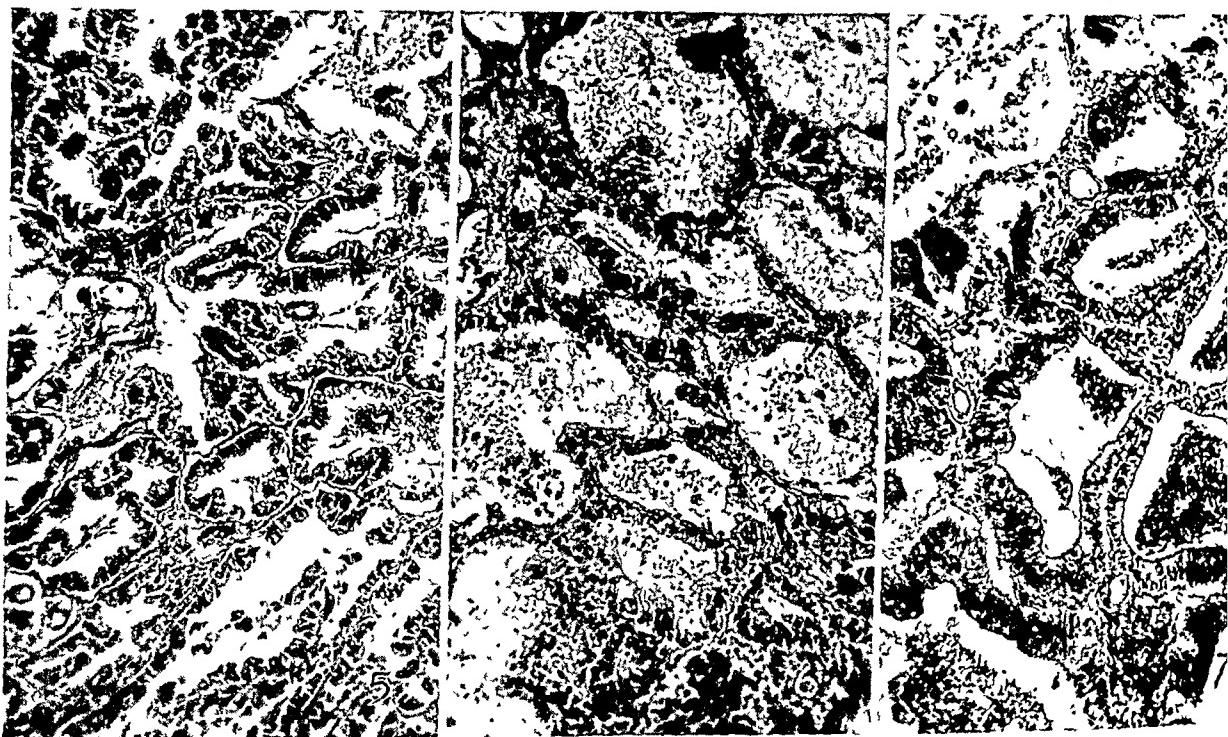


Fig. 5.—Section of the pulmonary metastases of the cancer illustrated in figure 4, showing alveolar septums regularly lined with one to several layers of cuboidal cells. Hematoxylin and eosin;  $\times 100$ .

Fig. 6.—Section from a tumor of the lung of a patient with mucoid adenocarcinoma of the pancreas, showing some of the alveoli lined with cuboidal cells and all of them filled with mucoid material. Most of those septums which with this magnification appear naked are nevertheless lined with remains of the epithelial cells that produced the secretion. Hematoxylin and eosin;  $\times 100$ .

Fig. 7.—Section from a tumor of the lung of a patient with adenocarcinoma of the colon, showing pulmonary alveoli regularly lined by one or several layers of columnar cells. There are numerous papillae projecting into the lumens. The lumens also contain some debris. Hematoxylin and eosin;  $\times 100$ .

fibrous tissue and tumor cells in glandular formation. Cancer cells were found in blood vessels, perivascular lymph channels and bronchioles. In addition to the areas grossly involved with carcinoma, microscopic sections disclosed tumor in the kidneys, the pericardium and lumbar vertebrae.

ANALYSIS OF 125 CASES OF CARCINOMA METASTASIZING  
TO THE LUNGS

In the last 6,000 consecutive autopsies at the Jefferson Medical College Hospital metastases of carcinoma, exclusive of primary pulmonary neoplasms, were noted in the lungs of 125 persons. The primary sites were in the skin, the sinuses, the breast, the mucous membranes of the mouth, the thyroid gland, the gastrointestinal tract, the liver, the gall-bladder, the pancreas, the adrenal gland and the genitourinary system. All histologic sections on file were examined with particular reference to whether the primary and the metastatic tumors had an adenomatous, a solid and compact or an anaplastic and loose arrangement of the cells. The cases were accordingly divided as follows: pure adenocarcinoma, 6 cases; mixture of solid and glandular carcinoma, 14 cases; mixture of anaplastic and glandular carcinoma, 8 cases; solid carcinoma, anaplastic carcinoma or mixtures of the two without adenocarcinoma, 97 cases. There were thus 28 cases in which there was glandular formation, but in only 6 of these was the primary tumor pure adenocarcinoma. These 6 cases were the only ones in which there was produced in the lungs a pure adenomatous formation with alveolar distribution of the cells. Of the remaining 22 cases in this group there were 3 in which the pulmonary metastatic distribution was of the alveolar and pneumonic type, 6 in which it was of the alveolar, pneumonic and irregular type and 13 in which the metastatic foci were of the pneumonic or irregular type or in which the collections of cancer cells were found only in blood vessels, perivascular lymphatic channels or the pleura and immediately subjacent lung. In the 97 cases in which no glandular formation was shown in the primary or the metastatic tumors the arrangement of the metastatic cancer in the lungs was irregular in some, pneumonic in others and in still others a combination of the two.

In the 6 cases in which pulmonary metastases with a pure alveolar distribution were observed, the carcinoma originated in the rectum in 2, in the colon in 2, in the pancreas in 1 and in the gallbladder in 1 (the case described in detail in the foregoing section). The gross distribution of the pulmonary metastases in these cases varied from a single nodule 5 mm. in diameter, in a case of carcinoma of the colon, to a multilobular and coalescing tumor replacing most of the parenchyma of the lung, in the case of carcinoma of the gallbladder. Microscopically (figs. 6 and 7) the cells lined the alveoli in one or several layers, produced papillae projecting into the lumens and were otherwise similar to the cells in the case of primary carcinoma of the gallbladder described in the foregoing section and typical likewise of those in cases of carcinoma described as originating in the pulmonary alveoli.

## COMMENT

A bronchiolar or bronchial origin of the tumor with an alveolar distribution has been repeatedly contested on the grounds that such a primary focus has never been demonstrated. I believe it is quite obvious that in the lung any origin in a relatively insignificant structure like a bronchiole would be impossible to find in the mass of cancer tissue always present when a fully developed tumor of this type is examined. In the larger and presumably older nodules, where the tumor probably originated, there are almost invariably foci of necrosis and liquefaction wherein not only the cancer tissue but the entire lung structure is destroyed. For this reason specimens showing the tumor fully developed are of little value in establishing the genesis of this particular tumor, and one must, therefore, turn to a specimen showing the neoplasm in a very early stage, encountered, perhaps, as an incidental finding, as in the first case reported here. In this instance there is no question that the structure lined in part with cuboidal and in part with transitional cells was a bronchiole, for bundles of smooth muscle were seen within its wall both in routine sections and in sections stained with Van Gieson's method. There is likewise no question that the attenuated or cuboidal epithelial cells lining almost all the alveolar septums of several surrounding microscopic fields originated in the bronchiolar epithelium, for in several areas the epithelial cells were seen to penetrate the bronchiolar wall and to be continuous with the alveolar lining cells. The diagnosis of carcinoma was based on (1) the variation in shape, size and staining qualities of some of the cells, (2) the presence of mitoses and (3) the presence of tumor cells within blood vessels.

The second case was described in detail because the lesions of the lungs were grossly and microscopically so typical of a primary "alveolar cell tumor" that had the original site in the gallbladder not been found the case would have been considered as one of primary pulmonary carcinoma. Although in the remaining cases a typical alveolar distribution of the cancer cells was disclosed microscopically, the gross appearance in 3 was less typical of "alveolar cell tumor" in that there were fewer pulmonary nodules and none coalesced or showed lobar consolidation as is sometimes seen in carcinoma primary in the lung. The explanation for this, however, is simple. Unlike the patient with carcinoma of the gallbladder, all 3 patients died from other causes before the pulmonary neoplasms could develop as they otherwise might have. Thus 1 patient died postoperatively from pulmonary embolism, 1 from intestinal obstruction and 1 from obstructive jaundice produced by a metastasis of the tumor in the hilar nodes of the liver. One of the remaining 2 patients who showed widespread pulmonary metastases died of ascites and

peritoneal carcinomatosis and the other of obstructive jaundice produced late in the disease.

A comparison of 125 cases of metastatic pulmonary carcinoma with over 100 cases of primary carcinoma of the lung disclosed that the distribution of the tumors in the lung was the same in each group. Grossly there were a few or many, sharply circumscribed or ill defined, single or confluent nodules, or there were diffuse areas resembling pneumonia irregularly distributed throughout the parenchyma. Microscopically, the distribution of the cancer appeared to depend entirely on the cell type, subject to the peculiarity of the framework of the organ itself. Thus in all tumors, whether primary or secondary in the lung, when the cancer cells were anaplastic and arranged loosely, or when they occurred in solid nests, or when they were in definite squamous cell formation, either they regularly occupied the alveolar spaces in the manner of an exudate in pneumonia or they replaced and entirely obliterated the pulmonary parenchyma. The former arrangement was more prevalent in the tumors unaccompanied by fibrosis, whereas the latter was more often seen in the tumors associated with a desmoplastic reaction. When the primary tumor was pure adenocarcinoma composed of cuboidal and columnar cells, the metastatic cells in the lungs lined the alveolar septa to produce the now often described alveolar cell arrangement. Finally, when the primary neoplasm was composed of a mixture of solid, anaplastic and adenomatous areas, the distribution in the lungs was also mixed but almost always a few cells lined the septa. In both primary and secondary tumors cancer cells were found in blood vessels, in perivascular and peribronchial lymph channels and in the lumens of bronchi and bronchioles.

In view of the foregoing facts it is obvious that any cancer composed of columnar or cuboidal cells in glandular formation will in the lungs line the septa and produce an alveolar arrangement of the cells. Since basal cells of the bronchi and the bronchioles not only are capable of giving rise to columnar or cuboidal cells in acinous formation but have been repeatedly seen to do so in cases in which a bronchial origin for carcinoma has been established, it is concluded that they form the starting point for the primary "alveolar cell tumor" and that there is no justification for assuming that the epithelial cells held to line alveolar septa are capable of giving rise to such a tumor. It is further concluded that in all pulmonary neoplasms showing this distribution of cells the alveolar septa are passive and act merely as a scaffolding on which the cancer cells grow.

#### SUMMARY AND CONCLUSION

Although reports of carcinoma considered to arise in epithelial cells lining the pulmonary alveoli continue to appear in medical literature,

there is still no satisfactory proof that septal cells are capable of giving rise to primary carcinoma.

In this report I have presented (1) a description of a very early "alveolar cell tumor" arising in a bronchiole and encountered as an incidental finding at autopsy, (2) a detailed description of a primary adenocarcinoma of the gallbladder with the classic gross and histologic alveolar cell distribution in the lungs and (3) an analysis of 125 cases of metastatic pulmonary carcinoma, exclusive of primary cancer of the lung. The macroscopic and microscopic distribution of the neoplasm in these cases of secondary carcinoma followed closely that of primary pulmonary carcinoma, of which over 100 cases have been studied for comparison. In each group when the primary tumor was pure adenocarcinoma the distribution in the lungs was that of an outright alveolar cell arrangement.

In view of these observations it is believed that all tumors presenting an alveolar cell arrangement in the lungs are secondary to recognized or unrecognized foci in other organs, or, if they are primary in the lungs, that they arise from the basal cells of the bronchi and bronchioles and that there is no justification for assuming that they originate in septal cells.

## EXPERIMENTAL NEPHROPATHIES

### VI. The Problem of Experimental Glomerulonephritis

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AND

JACK LANGE, M.D.

CHICAGO

IT IS relatively easy to produce selective, measurable injury of the epithelium of the renal tubules, as has been shown in previous papers in this series.<sup>1</sup> It is much more difficult to injure experimentally the glomeruli alone, or even the glomeruli and the tubules, without involving other organs. Faber<sup>2</sup> expressed the belief that the changes produced in the glomeruli by diphtheria toxin were equivalent "in a general way, to the intracapillary glomerulonephritis of Volhard and Fahr." Bell and Clawson,<sup>3</sup> after repeatedly injecting suspensions of living streptococci into a monkey over a period of four years, expressed the belief that they had "produced a form of chronic diffuse glomerulonephritis which resembles the 'parenchymatous' type of the human disease, but does not correspond to any human lesion in all respects." The nephrotoxic nephritis produced by Pearce,<sup>4</sup> Masugi,<sup>5</sup> Smadel<sup>6</sup> and Sarre<sup>7</sup> is said to resemble or, as reported by some, to be "identical clinically and pathologically" with human nephritis. However, the lack of success of most investigators, who have attempted to produce true glomerulonephritis is an indication of the difficulty inherent in the problem.

A study of spontaneous glomerulonephritis in man reveals numerous unit pathologic processes in the glomeruli: circulatory disturbances, such as ischemia, congestion, hemorrhage, thrombosis and embolism; degenerative changes, such as granular degeneration and fatty changes; exudative inflammation, with serous or cellular exudate in the capsular spaces; proliferation of the endothelium; formation of "epithelial crescents" and even complete fibrosis of glomeruli; swelling of the basement

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From the Department of Pathology, Northwestern University Medical School.

1. Simonds, J. P., and Hepler, O. E.: Arch. Path. **39**:103 and 133, 1945.
2. Faber, H. K.: J. Exper. Med. **26**:139, 1917.
3. Bell, E. T., and Clawson, B. J.: Am. J. Path. **7**:57, 1931.
4. Pearce, R. M.: Univ. Pennsylvania M. Bull. **16**:217, 1903-1904.
5. Masugi, M.: Beitr. z. path. Anat. u. z. allg. Path. **91**:82, 1933.
6. Smadel, J. E.: J. Exper. Med. **65**:527 and 541, 1937.
7. Sarre, H.: Deutsches Arch. f. klin. Med. **183**:515, 1939.

membrane, and complete necrosis. Some of these changes have been produced in experimental animals. In most cases of human glomerulonephritis, the unitary lesions in the glomeruli are accompanied by changes in the tubules and the interstitial tissue.

In order to account for the genesis of glomerulonephritis it is necessary to explain, first, the diffuse distribution of the lesions. Presumably the injurious agent reaches the kidneys by way of the blood stream. It should, therefore, affect all the glomeruli at the same time and to the same extent, since it reaches all the glomeruli in the same concentration. However, a study of sections of the kidneys involved in this disease in man reveals glomeruli in various stages of involvement; that is, the lesions are of different ages or duration.

The presence of lesions of different ages in the same kidney necessitates the explanation, second, of the temporary escape of some glomeruli from the effects of the injurious agent (that is, the injury of different glomeruli at different times) or of the differences in the rate of progress of the lesions in affected glomeruli. Richards and Schmidt<sup>8</sup> observed the capillaries in a glomerulus of the frog and illustrated changes in caliber extending over a period of forty-five minutes. Hayman and Starr,<sup>9</sup> using Nelson's<sup>10</sup> method, found that the percentage of glomeruli in the rabbit's kidney open to the circulation had a "normal" range between 42 and 100, with that in 10 of the 13 rabbits falling between 56 and 89. Moore and Hellman,<sup>11</sup> using the same method, found that the percentage of open glomeruli in control animals varied from 49 to 89, with an average of 63.8. It is evident, therefore, that there is an intermittency of glomerular circulation in the frog and the rabbit, probably controlled by changes in the arterioles at the point of origin of the capillaries. Whether a similar intermittency occurs in the dog and in man is uncertain, because glomerular activity is more constant in the latter animals than in the rabbit.<sup>12</sup> This intermittency of the glomerular circulation or, in its absence in some animals, "the differential tone of the afferent and efferent arterioles"<sup>12</sup> would protect some glomeruli temporarily from a circulating injurious agent. A colloidal nephrotoxic agent might be removed from the circulation by the reticuloendothelial cells before closed glomeruli became open to blood.

It is necessary to explain, third, the constant or the periodic presence of the injurious agent in the circulating blood. Since the nature and the

8. Richards, A. N., and Schmidt, C. F.: Am. J. Physiol. **71**:178, 1924.

9. Hayman, J. M., Jr., and Starr, I.: J. Exper. Med. **42**:641, 1925.

10. Nelson, B. T.: Anat. Rec. **23**:355, 1922.

11. Moore, R. A., and Hellman, L. M.: J. Exper. Med. **53**:303, 1931.

12. Smith, H. W.: The Physiology of the Kidney, New York, Oxford University Press, 1937, p. 249.

source of this agent are now unknown, it is not possible to account for its apparent irregular entrance into the blood stream.

It would also be desirable to know, fourth, whether the primary glomerular lesion is exudative or proliferative. Unless fibrin is present in appreciable amount and is precipitated in the capsular spaces, the exudative type of lesion usually heals, because the exudate is drained away down the tubules as rapidly as it is formed and does not accumulate to be ultimately organized. The proliferative type of lesion is more likely to end in destruction of the glomerulus involved.

The working hypothesis on which our experiments were based may be stated as follows: (1) A colloidal poison in the circulating blood will not pass through the normal glomerular filter but will be concentrated in its capillaries by loss of water and thus injure these structures; (2) a crystalloid poison in sufficiently high dilution will pass through the walls of the glomeruli without injuring them into the tubules where, by the absorption of water, it will reach a concentration capable of damaging the tubular epithelium. The second part of this hypothesis served admirably, the evidence for which has been presented in previous papers<sup>1</sup>; work based on the first part was not so successful. We later found that Faber had used the first part of this theory as a basis for his work.

#### METHOD

The method employed was similar to that described in connection with the effects of inorganic nephrotoxic agents.<sup>1</sup> Our object was to find a dose of a colloidal glomerular poison just sufficient to produce visible injury of the glomeruli without affecting other organs. For this purpose we used snake venoms, the toxin of the diphtheria bacillus and staphylococcus and streptococcus toxins. Sections of the kidneys were stained with hematoxylin and eosin, sudsan III and azocarmine and by Gamori's method for phosphatase. Similarly stained sections were made from the kidneys of normal control dogs for comparison.

#### THE EFFECTS OF SNAKE VENOMS ON THE KIDNEYS

Snake venoms have been used by a number of investigators as nephrotoxic agents. We used the venom of a rattlesnake (*Crotalus cerus*) and that of the water moccasin (*Agkistrodon piscivorus*), both of which act particularly on the endothelium of blood vessels.

Flexner and Noguchi<sup>13</sup> were among the first to study the effects of venoms on experimental animals. They discovered in *Crotalus* venom an endotheliolytic substance which they called "hemorrhagin." It causes vascular paralysis and capillary hemorrhages due not to simple rupture but to actual disappearance of the walls of the glomeruli as if by solution. Pearce<sup>14</sup> confirmed these observations and noted that when properly graded doses of the venom of *Crotalus*

13. Flexner, S., and Noguchi, H.: Univ. Pennsylvania M. Bull. 15:354, 1902-1903.

14. Pearce, R. M.: J. Exper. Med. 11:532, 1909.

adamanteus were given over a period of one to six days hemorrhage and exudate appeared in the capsular spaces of the glomeruli. In some instances the capillary endothelium seemed to disappear. Eisenbrey,<sup>15</sup> on the other hand, has stated that doses of rattlesnake venom that were large enough to produce renal lesions were so rapidly followed by death that it was not possible to study the functional capacity of the kidneys. With nonfatal doses, no visible renal damage could be identified, and the kidneys of these dogs given these doses excreted from 58.9 to 83.3 per cent of phenolsulfonphthalein. The only evidence of renal injury was a small amount of albumin in the urine. Essex and Markowitz<sup>16</sup> used a rattlesnake venom of such power that 0.4 cc. of a 2 per cent solution would produce in dogs a fall in blood pressure so precipitate as to kill. In such animals they observed, at autopsy, hemorrhages of the medullas and the pelvis of the kidneys and of the stomach, the intestines, the adrenal glands and the brain. Milles and co-workers<sup>17</sup> used a rattlesnake venom the minimal lethal dose of which was greater than 0.5 mg. per kilogram of body weight. In the kidneys of dogs poisoned with the venom they observed congestion of the glomerular and intertubular capillaries, with scattered hemorrhages and varying degrees of tubular degeneration. These changes were patchy in distribution, with intervening areas of relatively normal tissue. Taube and Essex<sup>18</sup> used lethal doses of rattlesnake venom that killed dogs in from eight minutes to twenty hours. In dogs that died in a few minutes, the glomeruli were massively congested, and the cells of Bowman's capsule and the tubular epithelium were markedly swollen. In animals that survived for a longer time, the chief changes in the kidneys appear to have been in the proximal convoluted tubular epithelium, which was desquamated, degenerated and necrotic. This change was found "particularly in the subcapsular zone" of the cortex. This indicates that the poison had escaped through the glomerular filter and had affected the first segment of the proximal convoluted tubules, that is, the same part of the renal units that is damaged by minimum necrotizing doses of potassium dichromate.<sup>1</sup> Also, hemorrhage occurred in both the medulla and the cortex. But all other organs, too, were found to be involved.

We injected moccasin venom into 10 dogs and rattlesnake venom into another 10 dogs intravenously. The doses varied from 0.1 to 0.5 mg. per hundred cubic centimeters of blood, and the animals received from one to six doses. Two dogs died in each group. The remaining 16 dogs were killed from one to five days after the first dose. Details of the findings in the dogs whose poisoning was fatal will be related briefly; the findings in the other dogs will be summarized.

Dog KSV 3 died six days after receiving the first of two doses of moccasin venom, each dose being 0.1 mg. per hundred cubic centimeters of blood. During this period the animal lost 19.5 per cent of its body weight. The urine contained albumin (3 plus). The glomeruli were only moderately engorged with blood, the endothelium was proliferated, the basement membrane was swollen, there were fine fat droplets in the hilar region in a few glomeruli, and the capsular spaces contained precipitated albuminous material and regurgitated necrotic tubular epithelium. The epithelium of the proximal convoluted tubules showed marked cloudy swelling and much necrosis that involved groups of cells rather than

15. Eisenbrey, A. B.: J. Exper. Med. **14**:462, 1911.

16. Essex, H. E., and Markowitz, J.: Am. J. Physiol. **92**:317 and 335, 1930.

17. Milles, G.; Müller, E. F., and Petersen, W. F.: Proc. Soc. Exper. Biol. & Med. **28**:561, 1931.

18. Taube, H. N., and Essex, H. E.: Arch. Path. **24**:43, 1937.

entire segments of the tubules. The phosphatase appeared normal in amount and distribution except that it had diffused into the cytoplasm of the necrotic cells. The liver and the adrenal glands showed striking congestion and some necrosis.

Dog KSV 18 died two days after receiving the first of two doses of moccasin venom, each dose being 0.5 mg. per hundred cubic centimeters of blood. This animal lost 10.35 per cent of its body weight. No albumin was demonstrated in the urine. The glomeruli were moderately congested, and many nuclei were pyknotic. The basement membrane was swollen, and the capsular spaces contained precipitate. The epithelium of the proximal convoluted tubules showed cloudy swelling and a moderate amount of necrosis. The phosphatase appeared to be normal in amount and distribution. The liver was severely congested with much central necrosis. The walls of many central veins were infiltrated with polymorphonuclear leukocytes and monocytes. Some of the hepatic cells were of giant size. There was extensive hemorrhage in the mucosa of the intestines and in the alveoli of the lungs.

Dog KSV 2 received 4 doses of rattlesnake venom, 2 doses were 0.1 mg. each, and 2 doses were 0.2 mg. each, per hundred cubic centimeters of blood. The animal died five days after the first dose. It had lost 27.6 per cent of its body weight. The urine was red (hemoglobin). The glomeruli were only moderately congested; many of their nuclei were pyknotic, and some were disintegrating; the basement membrane was swollen; many glomeruli contained considerable quantities of fat, and precipitate and regurgitated necrotic tubular epithelium were present in many capsular spaces. The epithelium of the first segment of the proximal convoluted tubules contained a good deal of fat and showed marked cloudy swelling and much necrosis, with red debris in the lumens. The phosphatase appeared to be somewhat reduced in amount and was diffused into the cytoplasm. The liver was fatty and markedly congested, and the central cells were necrotic. The adrenal glands were also congested and showed much necrosis.

Dog KSV 17 died twenty minutes after receiving one injection of rattlesnake venom, the dose being 0.5 mg. per hundred cubic centimeters of blood. The glomeruli were markedly congested and had some precipitate in the capsular spaces. The convoluted tubules appeared unchanged. The liver and the spleen were massively congested, and the red blood cells appeared to be clumped.

Lesions resulting from the action of the venoms in these 4 dogs were not limited to the kidneys. The liver and the adrenal glands were the organs most severely and constantly damaged. In the kidneys both glomeruli and tubules were injured.

The findings among the other 8 dogs poisoned with moccasin venom may be summarized as follows: Two of 5 dogs had albumin in the urine. Four of 5 were shown by the benzidine test to have blood (hemoglobin) in the urine. The renal phosphatase appeared to be normal in amount and distribution. Only 1 dog had easily visible fat in the glomeruli. In 3 dogs the glomeruli were essentially unchanged; in the others there was moderate congestion with varying degrees of endothelial proliferation. The epithelium of the proximal convoluted tubules of all dogs showed cloudy swelling, but in only 1 was there any noteworthy degree of necrosis. The loss of body weight in these 8 dogs varied from 0 to 12.60 per cent. In 3 of the 8 dogs other organs were involved as follows:

Dog KSV 5 received two doses of moccasin venom, each dose being 0.2 mg. per hundred cubic centimeters of blood, and was killed four days after the first dose. The liver and the spleen were definitely congested; there was some necrosis in the liver. The adrenal glands were unchanged.

Dog KSV 13 was given two doses of moccasin venom on the same day, each dose being 0.4 mg. per hundred cubic centimeters of blood, and was killed two days thereafter. This animal had hemorrhages in the mucosa of the stomach, in the interstitial tissue of the pancreas and in the triads of the liver.

Dog KSV 19 received two doses of moccasin venom, each dose being 0.5 mg. per hundred cubic centimeters of blood, and was killed two days later. There was hemorrhage in, and destruction of, many islets of the pancreas. The liver and the spleen were markedly congested, with areas of necrosis. In the liver the red blood cells appeared to be fused into compact masses. A lymph node was hemorrhagic, and there was pronounced phagocytosis of red blood cells in the sinusoids.

The lesions in the 8 dogs receiving rattlesnake venom may be summarized as follows: Two of 7 had albumin in the urine, and 2 of 4 were shown by the benzidine test to have blood (hemoglobin) in the urine. The renal phosphatase was normal in amount and distribution. Three dogs had easily visible fat in the glomeruli. In 2 dogs the glomeruli were definitely congested; in 3 there was hemorrhage and precipitate, and in 1 there was regurgitated necrotic epithelium in the capsular spaces. Endothelial proliferation was not marked. In all dogs in this group the epithelium of the proximal convoluted tubules showed cloudy swelling, but in only 2 was necrosis present in noteworthy amount. One dog had red blood cells in some of the tubules. The loss in body weight ranged from less than 1 to 9.75 per cent. Only 1 dog in this group of 8 showed noteworthy lesions in other organs. Dog KSV 6 received two doses of venom, each dose being 0.2 mg. per hundred cubic centimeters of blood, and was killed four days after the first dose. The liver and the spleen were congested, and the liver showed areas of necrosis. The adrenal glands were apparently normal.

The loss of weight of these dogs poisoned with snake venoms varied with the dose and the period of survival of the animal. Among those poisoned with moccasin venom, the greatest loss of weight was 19.5 per cent in six days. In this group: 6 lost from 2.16 to 19.5 per cent of their original body weight; 2 gained from 3.96 to 5.55 per cent; in 2 the weight remained approximately the same as at the beginning of the experiment. The greatest loss of weight among the dogs poisoned with rattlesnake venom was 27.6 per cent in five days. Nine of the 10 dogs in this group lost from 0.7 to 27.6 per cent, while the tenth animal died within twenty minutes after the administration of the venom. This loss of weight is an indication that the poison has far reaching effects on the entire body of the animal.

Four of 5 tested among the dogs poisoned with moccasin venom and 2 of 4 tested among those poisoned with rattlesnake venom were shown by the benzidine to have blood (hemoglobin) in the urine. This finding was probably the result of the known hemolytic effect of these two types of venom. The smallest dose of venom of each type to produce hemoglobinuria was 0.2 mg. per hundred cubic centimeters of blood.

Simonds and Brandes<sup>19</sup> found that the mean kidney weight-body weight ratio of normal dogs was 0.0057. The ratios for the 2 dogs that died after poisoning with moccasin venom were 0.0062 and 0.0078, respectively, with an average of 0.0070. The ratios for the other 8 dogs, in which the poison was not fatal during the period of the experiment, ranged from 0.0042 to 0.0058, with an average of 0.0047. The average ratio for the entire group of 10 dogs was 0.0052. The kidney weight-body weight ratios of the 2 dogs fatally poisoned with rattlesnake venom were 0.0037 and 0.0061, average 0.0049. The ratios of the other 8 dogs in this group ranged from 0.0047 to 0.0069, average 0.0055. The average

19. Simonds, J. P., and Brandes, W. W.: Arch. Path. 9:445, 1930.

for the entire group of 10 dogs was 0.0054. The cause of the marked relative increase in the weights of the kidneys of the dogs fatally poisoned with moccasin venom was apparently the accompanying high degree of cloudy swelling and necrosis of the proximal convoluted tubules, since the kidneys of these animals were not seriously congested. The relative increase in the weights of the kidneys of the dog that died within twenty minutes after injection of rattlesnake venom was due to the extreme degree of congestion of these organs.

The mean liver weight-body weight ratio of normal dogs was found by Simonds and Brandes<sup>19</sup> to be 0.0303. The ratios for the 2 dogs fatally poisoned with moccasin venom were 0.0329 and 0.0520, respectively, average 0.0425; the ratios for the other 8 dogs ranged from 0.0210 to 0.0332, average 0.0267. The average ratio for the entire group of 10 dogs was 0.0310. The liver weight-body weight ratios of the 2 dogs that died after poisoning with rattlesnake venom were 0.0269 and 0.0294, average 0.0282. Among the other 8 dogs in this group the ratios ranged from 0.0239 to 0.0341, average 0.0286. The average ratio for this entire group of 10 dogs was 0.0285. These ratios indicate that moccasin venom, particularly, produced a relative increase in the weight of the liver as a result of the accompanying severe congestion and necrosis.

#### THE EFFECTS OF DIPHTHERIA TOXIN ON THE KIDNEYS

Diphtheria toxin has been a favorite nephrotoxic agent, used by numerous investigators. Its effects vary with the dose and the time of survival of the animal. Lyon<sup>20</sup> used a toxin of such potency that 0.007 cc. killed rabbits in three to five days. Doses of 0.004 to 0.006 cc. might kill the animal in twelve days, while rabbits given 0.003 cc. usually survived but always had albumin and casts in the urine. Karsner and Denis<sup>21</sup> gave cats daily injections of 0.1, 0.25 and 0.5 unit of diphtheria toxin for periods up to twenty-seven days. With the smallest dose albuminuria was present after the seventh day; with larger doses it appeared earlier. Faber<sup>2</sup> thought that the lesions produced by diphtheria toxin corresponded in a general way to the intracapillary glomerulonephritis of Volhard and Fahr. With properly graded doses it is usually possible to injure the kidneys of an animal without producing visible effects in other organs. Diphtheria toxin injected intravenously disappears quickly from the blood. Bieling and Gottschalk<sup>22</sup> found more than half of the injected toxin in the abdominal organs, particularly in the spleen. Speransky<sup>23</sup> asserted that shortly after introduction into the blood stream a small amount of diphtheria toxin is excreted unchanged by the kidneys.

Human patients with diphtheria frequently have albumin and casts in their urine, and various lesions are found in their kidneys. Earlier investigators (Cornil and Brault<sup>24</sup>; Langhans<sup>25</sup>; Oertel<sup>26</sup>) record damage of the tubular epithelium as the most frequent and severe lesion found in the kidneys of patients dead from diphtheria. The predominant lesion in 117 of 171 cases of fatal

20. Lyon, G.: J. Path. & Bact. **9**:400, 1904.

21. Karsner, H. T., and Denis, W.: J. M. Research **19**:270, 1914.

22. Bieling, R., and Gottschalk, A.: Ztschr. f. Hyg. **99**:125 and 142, 1923.

23. Speransky, A.: Ann. Inst. Pasteur **41**:1063, 1927.

24. Cornil, A. V., and Brault, A.: Études sur la pathologie du rein, Paris, F. Alcan, 1884, p. 149.

25. Langhans, T.: Virchows Arch. f. path. Anat. **99**:193, 1885.

26. Oertel, M. J.: Die Pathogenese der epidemischen Diphtherie nach ihrer histologischen Begründung, Leipzig, F. C. W. Vogel, 1887.

diphtheria studied by Councilman, Mallory and Pearce<sup>27</sup> was that of the tubules; in 43 cases there was interstitial nephritis characterized by infiltration of the intertubular tissues with plasma cells and lymphocytes; in the remaining 11 cases acute glomerulonephritis was the chief lesion. One of us<sup>28</sup> studied 7 cases of fatal diphtheria and found severe damage of both glomeruli and tubules in all cases. It is evident from this brief review of the literature that in man diphtheria toxin absorbed from the primary site of the disease injures predominantly the tubular epithelium. This may be due in part to the greater ease with which injury of the tubules can be detected and in part to the relatively small size of the colloidal molecule of the toxin, which allows it to escape through glomeruli that have been only slightly damaged by this agent.

Numerous pathologic processes observed in the glomeruli of experimental animals after injection of diphtheria toxin have been described. They include: congestion (Lyon<sup>20</sup>; Faber<sup>2</sup>); thrombosis, often hyaline in nature (Karsner and Denis<sup>21</sup>; Bailey<sup>29</sup>; Lyon<sup>20</sup>; Frothingham<sup>30</sup>); increase in polymorphonuclear leukocytes (Flexner<sup>31</sup>; Lyon<sup>20</sup>; Bailey<sup>29</sup>; Schlayer and Hedinger<sup>32</sup>; Takayasu<sup>33</sup>); proliferation of the endothelium of the glomerular capillaries (Faber<sup>2</sup>; Karsner and Denis<sup>21</sup>; Schlayer and Hedinger<sup>32</sup>; Takayasu<sup>33</sup>); swelling and degeneration of the glomerular epithelium (Lyon<sup>20</sup>); hyaline degeneration (Lyon<sup>20</sup>); fatty degeneration (Bailey<sup>29</sup>; Lange<sup>28</sup>); precipitation in the capsular space (Faber<sup>2</sup>; Karsner and Denis<sup>21</sup>; Schlayer and Hedinger<sup>32</sup>; Takayasu<sup>33</sup>); necrosis of the glomeruli (Flexner<sup>31</sup>; Faber<sup>2</sup>; Bailey<sup>29</sup>); mitotic activity in the glomerular endothelium (Flexner<sup>31</sup>); formation of "epithelial crescents" (Takayasu<sup>33</sup>).

A smaller number of pathologic processes have been described in the tubules: fatty degeneration (Bailey<sup>29</sup>; Lyon,<sup>20</sup> who calls attention to the necessity of using an animal which does not normally have fat in its tubular epithelium); cloudy swelling (Lyon<sup>20</sup>; Karsner and Denis<sup>21</sup>; Schlayer and Hedinger<sup>32</sup>; and others); necrosis of the tubular epithelium (Karsner and Denis<sup>21</sup> and others observed this change chiefly in the proximal convoluted tubules, while Lyon<sup>20</sup> found it most marked in the ascending limb of Henle's loop); deposition of granular debris in the lumens of the tubules (Lyon,<sup>20</sup> and others) casts (Lyon<sup>20</sup>; Bailey,<sup>29</sup> and others), and sometimes blood casts. Calcification is mentioned only by Flexner.<sup>31</sup>

In our experiments we injected diphtheria toxin intravenously into 21 dogs, the doses being equivalent to 0.6 and 0.8 minimum lethal dose (M.L.D.) per hundred cubic centimeters of blood. Two animals, because of a mistake in calculation, were given six times the minimum lethal dose. The dogs were given one to eight doses and were killed from one to four days after the first dose. Some of the animals were therefore given as many as three injections in one day.

27. Councilman, W. T.; Mallory, F. B., and Pearce, R. M.: J. Boston Soc. M. Sc. 5:139, 1900-1901.

28. Lange, J.: A Study of the Effects of Diphtheria Toxin (An Example of a Colloidal Poison) on the Kidney, Thesis, Northwestern University Graduate School, 1942.

29. Bailey, C. H.: J. Exper. Med. 25:109, 1917.

30. Frothingham, C.: J. M. Research 30:365, 1914.

31. Flexner, S.: Bull. Johns Hopkins Hosp. 6:259, 1897.

32. Schlayer and Hedinger: Deutsches Arch. f. klin. Med. 90:1, 1907.

33. Takayasu, H.: Deutsches Arch. f. klin. Med. 92:127, 1908.

Congestion of the glomeruli was striking and widely diffuse in some dogs; in others it was more irregular, involving only some of the glomeruli or even parts of the glomeruli. Many glomeruli were large, completely filling the capsular spaces. In some this was due to congestion; in others, to proliferation of the endothelium of the glomerular capillaries. The glomeruli that had the greatest amount of endothelial proliferation showed the least amount of congestion. In 1 dog (KDph 2) that received six times the minimum lethal dose the glomeruli were small because they were compressed by the large amount of material in the capsular spaces (fig. 1). In most of the glomeruli the basement membrane was swollen. The degree of swelling appeared to be proportional to the size of

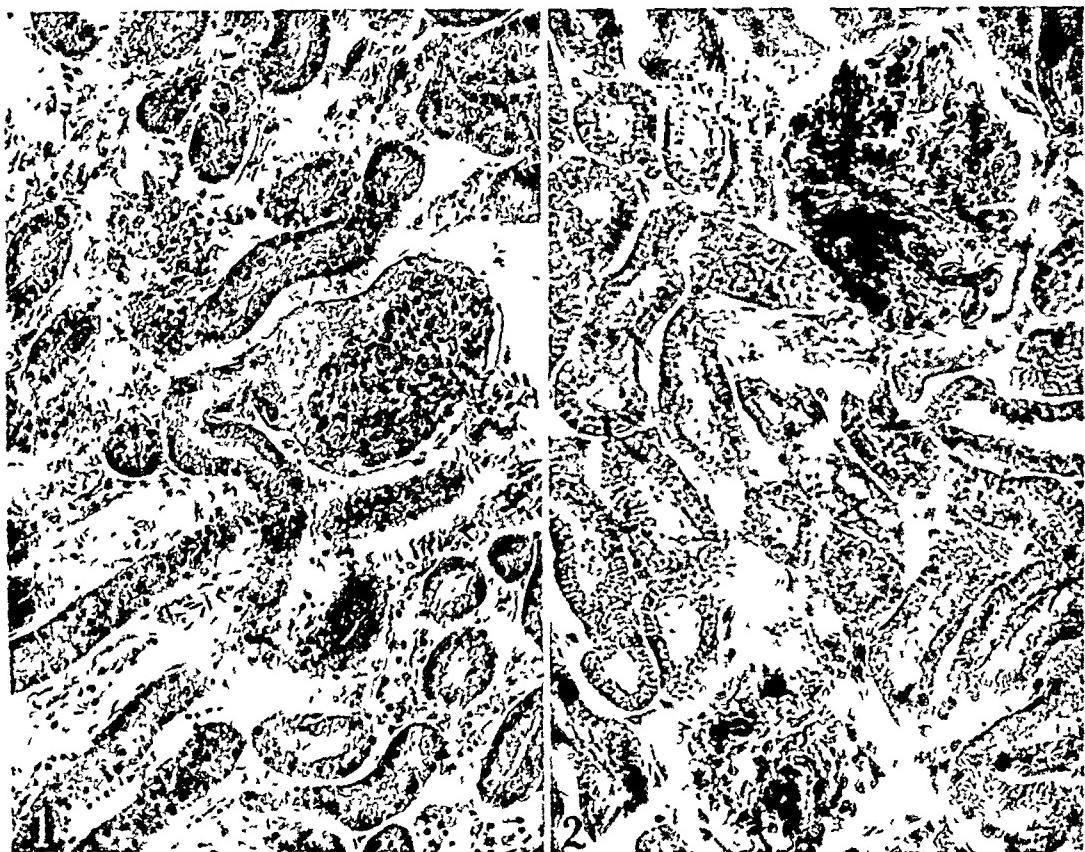


Fig. 1.—Section of kidney from dog KDph 2, which received six times the minimum lethal dose of diphtheria toxin per hundred cubic centimeters of blood and died on the third day thereafter. The capsular space is filled with precipitated protein. The nuclei of the glomeruli and of the tubules are pyknotic. The tubular epithelium is swollen and granular, and some cells are necrotic.  $\times 155$ .

Fig. 2.—Section of kidney from dog KDph 3, which received 0.6 M.L.D. of diphtheria toxin per hundred cubic centimeters of blood and was killed forty-eight hours later. Most of the glomeruli contained much fat (black in the photomicrograph).  $\times 155$ .

the dose, although in only 2 of the 9 instances in which the dose was 0.8 M.L.D. were sections of the kidneys stained with azocarmine. Fat in quite large amounts was seen in the glomeruli of 5 dogs (fig. 2). The proportion was higher among those receiving 0.8 M.L.D. The albuminous material precipitated in the capsular spaces was scanty except in 1 dog that received one dose six times

the minimum lethal dose and died four days later. In this animal practically every capsular space was filled with this material. In many glomeruli the nuclei were quite pyknotic. Small blue-black masses resembling chromatin liberated from nuclei by karyorrhexis were also present in many glomeruli. These were more numerous in the kidneys of dogs given the larger doses of toxin. In these dogs there were hemorrhages in the gastric mucosa, the serous membranes and the adrenal glands and central necrosis in the liver.

In all the dogs of this group the epithelium of the proximal convoluted tubules showed cloudy swelling. In some animals the swelling was so marked that the lumen was almost obliterated. In others, the lumens contained much granular debris, but no definite casts were observed. Fatty changes were observed in the part of the proximal convoluted tubules that lay within the labyrinth in 10 dogs. This is probably significant, since fat is not present in this portion of the proximal convoluted tubules in normal dogs. Its presence in the terminal portion of these tubules has no pathologic significance.<sup>1</sup> Necrosis was found in many proximal convoluted tubules. It tended to involve single cells or groups of cells rather than entire segments of tubules as in the kidneys of dogs poisoned with crystalloid, inorganic agents. It did not have any characteristic location but appeared to occur chiefly in the upper segments. We did not observe necrosis in Henle's loop.<sup>20</sup> Sections of the kidneys of 11 dogs were stained by Gomori's method. In general, phosphatase was present in normal amount and distribution. In a few instances there was a slight tendency to diffuse into the cytoplasm of the cells.

Albuminuria was not constantly observed. Only 10 of the 20 dogs examined were found to have albumin in their urine. Casts were present in a slightly larger number, namely 14. The kidney weight-body weight and liver weight-body weight ratios increased with the size of the dose. This indicates a greater degree of injury of the kidneys with increase in the size of the dose. The relative increase in the weights of the kidneys and the liver was due in part to congestion and in part to cloudy swelling and necrosis. No changes of significance were observed in the interstitial tissues. In a few dogs there was some congestion of the intertubular capillaries. But no distinct alterations could be seen in these structures.

#### THE EFFECTS OF STAPHYLOCOCCUS TOXIN ON THE KIDNEYS

Staphylococcus aureus produces a toxin that has leukotoxic, hemolytic and tissue-necrotizing<sup>34</sup> effects. The three components are protein in nature, as each gives rise to antibodies when injected into animals. The amount of each factor varies in the toxins of different strains of staphylococci. Julianelle<sup>35</sup> stated that all pathogenic strains produce hemolysin. The tissue-necrotizing toxin has been studied by Gross,<sup>36</sup> by Burnet<sup>37</sup> and by Bigger and his co-workers.<sup>38</sup>

A dose of staphylococcus toxin can be selected that will limit its effects to the kidneys without producing visible injury in other organs of the body. It should be a toxin in which the necrotizing factor exceeds the leukotoxic and

34. Gay, F. P.: Agents of Disease and Host Resistance, Springfield, Ill., Charles C Thomas, Publisher, 1935.

35. Julianelle, L. A.: J. Infect. Dis. **31**:256, 1922.

36. Gross, H.: Centralbl. f. Bakt. (Abt. 1) **115**:367, 1930.

37. Burnet, F. M.: J. Path. & Bact. **32**:717, 1929; **33**:1, 1930; **34**:471, 1931.

38. Bigger, J. W.; Bland, C., and O'Meara, R. A.: J. Path. & Bact. **30**:261, 1927.

hemolytic factors. It is more difficult to select a dose that will act only on the glomeruli without producing extensive injury of the tubular epithelium. Von Glahn and Weld<sup>39</sup> used rabbits and cats and concluded that staphylococcus toxin damages the kidneys selectively and that its primary site of action is the glomerular capillaries and other small blood vessels. The extent of the damage is proportional to the amount of toxin administered. They described the following changes occurring in the kidneys after poisoning with staphylococcus toxin:

1. The earliest visible change is an extraordinary distention of the glomerular capillaries.
2. With more extensive damage, the endothelial lining of the glomerular capillaries disappears, and a layer of fibrin covers the inner surfaces of the capillary loops.
3. A precipitate of albuminous material is formed in the capsular spaces although these spaces are almost completely filled with the extremely congested glomeruli.
4. The congestion extends from the glomeruli backward along the course of their blood supply through the afferent arteries to the intralobular arteries, frequently with complete necrosis of the walls of the arteries.
5. Accompanying these changes in the glomeruli and blood vessels, changes in the epithelium of the proximal convoluted tubules range from cloudy swelling and fine granulation of the cytoplasm to necrosis that may be so extensive as to involve the greater part of the cortex.

Rigdon and his co-workers<sup>40</sup> agreed with Von Glahn and Weld as to the essential details of the renal effects of this toxin and add that extensive thrombosis accompanies the damage to the glomerular capillaries and small arteries. Rigdon<sup>41</sup> used dogs and rabbits and concluded that the renal effects of staphylococcus toxin may be conveniently divided into two groups, namely, hemorrhagic and necrotic. The hemorrhagic type was more frequently encountered in dogs and the necrotic type in rabbits. However, he observed that the lesions are influenced by the quantity of toxin, the method of administration and the length of time elapsing before death. The hemorrhagic and necrotic areas suggested that the toxin either injures the endothelium of the capillaries, causing thrombosis and infarction, or injures the epithelial cells by acting directly on these cells. The dog appears to be more susceptible to the action of this toxin than does the rabbit, hence it is possible that the differences in the types of lesions found in the kidneys of the dog may be due to the earlier death of this animal. Rigdon and his associates<sup>40</sup> were unable to demonstrate a sufficient number of thrombi to consider the necrosis secondary to obstruction of blood vessels. They concluded, therefore, the toxin acts directly on the tubular epithelium and is the chief cause of the extensive necrosis observed.

Massive cortical necrosis as a result of the action of staphylococcus toxin was known many years before the work of the investigators quoted in the preceding paragraph. According to Neisser and Levaditi<sup>42</sup> and Neisser and Wechsberg,<sup>43</sup> the necrosis is equivalent to infarction. Rigdon and his co-workers<sup>40</sup> commented on the difficulty of differentiating quantitatively between the necrosis which is the result of the circulatory disturbances and that which is due to the direct action of the toxin when it comes in contact with the tubular epithelium

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- 39. Von Glahn, W. C., and Weld, J. T.: *J. Exper. Med.* **61**:1, 1935.
  - 40. Rigdon, R. H.; Joyner, A. L., and Ricketts, E. T.: *Am. J. Path.* **10**:425, 1934.
  - 41. Rigdon, R. G.: *Arch. Path.* **20**:201, 1935; *Arch. Int. Med.* **57**:117, 1936.
  - 42. Neisser, M., and Levaditi, C.: *Compt. rend. Cong. internat. de méd. (Sect. de path. gén. et de path. expér.)*, 1900, p. 475.
  - 43. Neisser, M., and Wechsberg, F.: *Ztschr. f. Hyg. u. Infektionskr.* **36**:299, 1901.

after it has escaped through the damaged glomerular filter into the lumens of the tubules. Rigdon<sup>41</sup> concluded that both factors are concerned in the process of necrosis. He also studied the lesions in the kidneys of 7 patients who died from staphylococcal infections and noted in the kidneys of some of them changes which resembled those observed in experimental animals.

In our experiments we attempted to find a dose of staphylococcus toxin that would produce minimal lesions in the kidneys of dogs without causing the extensive hemorrhages and the massive necrosis observed by Rigdon<sup>41</sup> and others. Twelve dogs were given multiple (2 or 3) daily intravenous doses of the toxin, the doses being 0.06 and 0.07 cc. per hundred cubic centimeters of blood. Altogether, from two to seven doses were administered, and the animals were killed in from one to four days after the first dose. These doses affected both glomeruli and tubules but did not induce extensive hemorrhages or massive necrosis.

Since we used only small doses, the changes differed from those reported by others in the extent, rather than in the character, of the lesions, and it is not necessary to describe them in detail. The most constant changes observed in our dogs were marked congestion of the capillaries and swelling of the basement membrane of the glomeruli and cloudy swelling and necrosis of the epithelium of the proximal convoluted tubules. Although the capillaries of the glomeruli were excessively distended with blood, the amount of precipitate in the capsular spaces was minimal, and no red blood cells were seen either in the capsular spaces or in the lumens of the tubules. Definite, but only occasional, thrombi were found in the glomerular capillaries. These were observed more frequently in the dogs that received the larger doses, but there was no constant relation to the number of doses. The basement membrane of the glomeruli was swollen in all 7 dogs sections of whose kidneys were stained with azocarmine. The swelling and irregularity of the membrane were more pronounced in dogs that received the larger doses. In most of these dogs there was definite, but often irregular, proliferation of the endothelium of the glomerular capillaries. The proliferation was most marked in the region of the hilus of the glomerulus involved. It was often limited to a single loop. In some glomeruli there were one or more huge, moderately pyknotic oval or indented nuclei. Disintegrating nuclei appeared as one to several dark blue granules. Polymorphonuclear leukocytes were not noticeably increased in the glomeruli above the number that would be expected in the increased quantity of blood. In 2 dogs fat in considerable amount was visible in many glomeruli.

In all dogs, regardless of the size or the number of the doses, the epithelium of the proximal convoluted tubules showed marked cloudy swelling. In some tubules the epithelium was merely swollen and the lumen almost obliterated. In others the inner margins of the cells were irregular and ragged, while the lumens were fairly large and contained granular material. No definite casts were observed in any of the tubules, although in some the material in the lumens was compact and granular. Some degree of necrosis of the epithelium of the proximal convoluted tubules was observed in all dogs. The necrosis rarely involved all the cells in any segment of a tubule. Rather, it involved groups of cells. The appearance of the necrotic cells varied. Some were entirely without visible nuclei; in others the nuclei were visible but pale and shadowy; in still others the nuclei were broken up into fragments or appeared as a single, small, compact mass of blue-black chromatin. The necrosis was sometimes present in, or entirely

limited to, the zone between the renal capsule and the first row of glomeruli. This indicates that the toxin was present in sufficient strength to affect the cells in the first segment of the proximal convoluted tubules.

#### THE EFFECTS OF STREPTOCOCCUS TOXIN ON THE KIDNEYS

The frequency with which the kidneys are involved in scarlet fever, erysipelas and other streptococcal infections, particularly those of the upper respiratory tract,<sup>44</sup> led us to attempt to injure the glomeruli with streptococcus toxin. Mallory and Keefer<sup>45</sup> found the kidneys involved in 24 (54 per cent) of 44 cases of scarlet fever and in 8 (36 per cent) of 22 cases of fatal erysipelas. But the lesions appear to have been entirely of the interstitial type, characterized by presence of lymphocytes and plasma cells between the tubules and about the glomeruli, as originally described by Councilman.<sup>46</sup> Brody and Smith<sup>47</sup> found lesions in the kidneys in 32 (72.6 per cent) of 44 cases of scarlet fever. In only 1 of these cases was typical glomerulitis shown; in the remainder the involvement was of the interstitial type. In 8 other cases "some histologic suggestion of very early glomerular changes was seen." They commented that perhaps a partial explanation of the comparative rarity of the glomerular type of lesion is that more than three quarters of their patients died before the twenty-first day of their illness, before the glomerular type of nephritis ordinarily appears.

Living and killed streptococci and various products derived from these organisms have been used by different investigators in attempts to injure the glomeruli. Bell, Clawson and Hartzell<sup>48</sup> injected live cultures of "streptococci from human lesions" into rabbits and monkeys. In the rabbits they found only "the ordinary interstitial type, i. e., lymphocytic interstitial nephritis." With 14 monkeys used, severe renal lesions were produced in only 5: nephrosis in 2; acute interstitial nephritis in 1, and acute glomerulonephritis in 1; the fifth monkey was killed after four years of repeated injections of living streptococci and was found to have "chronic diffuse glomerulonephritis which resembled the human disease but not in all respects."<sup>3</sup> Stoddard and Woods<sup>49</sup> injected powdered washed streptococci into rabbits and produced vacuolar degeneration of the renal tubules, giving the cells the appearance of fatty changes, up to the collecting tubules. Lukens and Longcope,<sup>50</sup> using rabbits and injecting heat-killed streptococci directly into one renal artery, produced glomerulonephritis, sometimes focal, sometimes diffuse.

Brody and Smith<sup>47</sup> expressed the belief that the evidence presented by them suggests that the lesions which they described were the result of the circulation of a toxin. Mallory and Keefer,<sup>45</sup> on the other hand, held that the late tissue reactions observed in streptococcal infections are the response of the host to the infection and that they are the result in part of an antigen-antibody reaction rather than of direct toxic damage of the tissues. The fact that nephritis usually develops only after the acute symptoms of scarlet fever have subsided suggests that the lesions in the kidneys are related to an antigen-antibody reaction. The

44. Seegal, D.; Seegal, B. C., and Lyttle, J. D.: J.A.M.A. **105**:17, 1935.

45. Mallory, G. K., and Keefer, C. S.: Arch. Path. **32**:334, 1941.

46. Councilman, W. T.: J. Exper. Med. **3**:393, 1898.

47. Brody, H., and Smith, L. W.: Am. J. Path. **12**:373, 1936.

48. Bell, E. T.; Clawson, B. J., and Hartzell, T. B.: Am. J. Path. **1**:247, 1925.

49. Stoddard, J. L., and Woods, A. C.: J. M. Research **34**:343, 1916.

50. Lukens, F. W. D., and Longcope, W. T.: Bull. Johns Hopkins Hosp. **53**:511, 1931.

soluble toxin found in broth cultures of streptococci is antigenic and therefore probably a protein. This was shown for the streptococcus of erysipelas by Birkhaug<sup>51</sup> and for the streptococcus of scarlet fever by Dick and Dick.<sup>52</sup> If the antigen (streptococcus toxin) is a colloid, it appears possible that it might, when injected into the circulating blood in sufficient amount and concentrated in the glomeruli, directly injure this part of the renal units.

We injected streptococcus toxin into 6 dogs. Four of these dogs were given from two to six doses of the toxin of the streptococcus of erysipelas, the doses being 0.2 and 0.25 cc. per hundred cubic centimeters of blood. The other 2 dogs received 6 doses each of the toxin of the streptococcus of scarlet fever, the doses being 2,000 and 5,000 cutaneous test doses, respectively, per hundred cubic centimeters of blood. The animals were killed from one to four days after the administration of the first dose. Of these 6 dogs, 5 had albumin in the urine, ranging from a trace to 2 plus.

The glomeruli of 2 dogs contained marked amounts of fat, and in the same animals there was fat in the upper segments of the proximal convoluted tubules. In all these dogs there was engorgement of the glomerular capillaries, but this was prominent in only 3. Proliferation of the endothelium of the glomerular capillaries was slight or questionable. In 4 dogs there was some disintegration of nuclei in the glomeruli. Cloudy swelling of the tubular epithelium was present in all dogs of this group but was marked in only 2. In the latter 2 dogs there was a moderate amount of necrosis of the tubular epithelium. In 2 dogs the intertubular capillaries were congested. In the kidneys of 2 dogs, one injured with the toxin of the streptococcus of erysipelas, and the other with that of the streptococcus of scarlet fever, foci of interstitial lymphocytic and plasma cell infiltration were found.

In all these dogs the changes described were moderate in degree but those in the animals given erysipelas streptococcus toxin were definitely more prominent. Five thousand cutaneous test doses of the scarlet fever streptococcus toxin produced more injury in the kidneys than did 2,000 such doses. The small number of dogs and the limited variation in the dosage employed do not warrant drawing definite conclusions concerning the effects of streptococcus toxin on the kidneys. Our results are presented as indicating the possibilities and the limitations of streptococcus toxin as a nephrotoxic agent.

#### COMMENT

Theoretically, a colloidal poison introduced into the circulating blood in optimal doses might be expected to become sufficiently concentrated, through loss of water by filtration, to injure the glomeruli. The chief obstacles to the successful production of experimental glomerulonephritis arise from the lack of a colloidal poison ideal for this purpose and from the difficulties inherent in identifying and accurately evaluating the changes produced in the glomeruli.

Snake venoms are complex substances. They consist, at least in the crude form, of proteins (both albumins and globulins), lipoproteins, proteoses and peptones, mucin and mucin-like substances, detritus of

51. Birkhaug, K. E.: Bull. Johns Hopkins Hosp. **36**:248, 1925; **37**:85, 1926.

52. Dick, G. F., and Dick, G. H.: J.A.M.A. **82**:265 and 544, 1924.

cells and various salts.<sup>53</sup> Micheel and Kraft<sup>54</sup> expressed the belief that native venoms consist largely of proteins and related substances which do not pass through cellophane. But they also considered the possibility that the poisonous part of venom might be a prosthetic group that requires a colloidal carrier. Peck and Marx<sup>55</sup> found that the hemorhagin and the hemolysin are in the protein fraction of venom and that as regards cellophane they are practically nondialyzable.

Snake venom, therefore, appears to meet one of the requirements of a colloidal poison that might be used to produce experimental glomerulonephritis. However, as shown in our experiments, the constant damage of the tubular epithelium that equaled or exceeded the visible injury of the glomeruli, the intravascular hemolysis indicated by the frequent presence of hemoglobinuria, the definite and sometimes striking loss of body weight, the changes in the liver weight-body weight ratios in our dogs and the narrow range between the minimum lethal dose and the nephrotoxic dose as pointed out by Eisenbrey,<sup>16</sup> all indicate that snake venom is not suitable for the production of experimental glomerulonephritis.

Diphtheria toxin was held by Pearce<sup>56</sup> to be the best of the agents thus far used to produce glomerular damage. But it lacks much of being an ideal agent. It damages the tubules as much as, or more than, it injures the glomeruli. A dose of this toxin that will visibly injure the kidneys will also affect other organs, as shown by the increase in liver weight-body weight ratios mentioned in a preceding paragraph. The action of diphtheria toxin is not as constant, its effects cannot be measured with the same degree of accuracy, the results of its injection cannot be predicted with as much certainty, and the changes produced are not as distinct and definite as those produced by crystalloid, inorganic agents used on other dogs in this series of experiments.

A similar objection applies to staphylococcus toxin. It is such a powerful epithelial poison that it is difficult to select a dose that will injure the glomeruli without at the same time producing extensive necrosis of the tubular epithelium. The molecule of this toxin, although undoubtedly protein since it induces the formation of antibodies, is apparently relatively small and passes through an even slightly damaged glomerular filter. This is the probable explanation of the extensive

53. Do Amoral, A.: Snake Poisoning, in Nelson's Loose Leaf Medicine, New York, Thos. Nelson & Sons, 1932, vol. 2, chap. 5, p. 683.

54. Micheel, F. and Kraft, K., cited by Medes, G., in Luck, J. M.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1939, vol. 8, p. 196.

55. Peck, S. M., and Marx, W. J.: J. Mt. Sinai Hosp. 6:171, 1941.

56. Pearce, R. M.: Arch. Int. Med. 5:133, 1910.

necrosis of the tubular epithelium with which it comes in contact in a concentrated form because of absorption of water.

In view of the frequency with which glomerulonephritis seems to follow streptococcal infections, efforts to injure the glomeruli with streptococcus toxin have been particularly disappointing. The renal changes induced were relatively minor.

Numerous chemical agents in adequate doses will injure the glomeruli. Even crystalloid poisons, such as uranyl nitrate, if present in the plasma in sufficient concentration (3 mg. or more per hundred cubic centimeters of blood) will cause visible damage to the glomeruli. Cantharidin has been frequently used as a glomerular poison, but this substance also injures the tubules.<sup>57</sup> If the glomeruli are to be injured by a poison concentrated in the plasma through loss of water by filtration, the poison must be colloidal in nature. The colloidal poison ideal for this purpose must have at least four characteristics or qualities:

1. The molecule of the ideal colloidal nephrotoxic agent must be of a size to prevent its passage through the glomerular filter. Little or nothing is known concerning the size of the smallest molecule that is retained by the glomerular filter. Phosphatase, with a molecular weight between 6,000 and 10,000,<sup>58</sup> and inulin, with a molecular weight of 5,000<sup>59</sup> but with a diffusion coefficient equivalent to a molecular weight of 15,000,<sup>60</sup> pass readily through the walls of the glomeruli. We have no adequate knowledge concerning the size of the molecules of the colloidal poisons used in our experiments. We believe that all of them are colloids and protein in nature because each of them will induce the formation of antibodies when injected into an animal. Gralen and Svedberg<sup>61</sup> found the molecular weight of purified crotoxin, the neurotoxic venom of *Crotalus terrificus*, to be about 30,000. Patermann and Pappenheimer<sup>62</sup> determined the molecular weight of diphtheria toxin to be 74,000. The molecular weight of Lancefield's<sup>63</sup> toxic "M substance" of streptococcus was found to be 41,000.<sup>64</sup> If the colloidal poisons used by us had molecular weights no greater than those mentioned, their molecules were

57. MacNider, W. DeB.: Physiol. Rev. **4**:595, 1924. Folin, O.; Karsner, W. T., and Denis, W.: J. Exper. Med. **16**:789, 1912. Eisenbrey,<sup>15</sup> Lyon.<sup>20</sup>

58. Albers, H., and Albers, E.: Ztschr. f. physiol. Chem. **232**:165 and 189, 1935.

59. Westfall, B. B., and Landis, W. M.: J. Biol. Chem. **116**:727, 1936.

60. Bunim, J. J.; Smith, W. W., and Smith, H. W.: J. Biol. Chem. **118**:667, 1937.

61. Gralen, N., and Svedberg, T.: Biochem. J. **32**:1375, 1938.

62. Patermann, M. L., and Pappenheimer, A. M., Jr.: J. Physiol. Chem. **45**:1, 1941.

63. Lancefield, R. C.: J. Exper. Med. **47**:91, 843 and 859, 1928.

64. Pappenheimer, A. M., Jr.; Williams, J. W., and Zittle, C. A.: J. Immunol. **43**:61, 1942. Zittle, C. A., and Mudd, S.: Ann. New York Acad. Sc. **43**:47, 1942.

small in comparison with those of other proteins, such as serum albumin. Even very large molecules of foreign proteins, such as egg albumin, when introduced into the circulation are, it is known, excreted by the glomeruli. But Babcock<sup>65</sup> has shown that such foreign proteins first injure the glomeruli and thus increase the permeability of these structures before being excreted.

2. The toxicity of the ideal colloidal nephrotoxic poison must be such that an amount introduced into the circulating blood which will not damage other organs will be sufficiently concentrated in the glomeruli through loss of water by filtration to produce local injury. That is, there must be a wide range between its minimum nephrotoxic dose and its minimum lethal dose. Ricin, a vegetable protein with a large molecule, is far too toxic to be an ideal colloidal poison for this purpose.<sup>66</sup> In our experience rattlesnake and moccasin venoms possess the same objectionable quality.

3. The ideal colloidal nephrotoxic poison must not produce undesired side effects. Some of these effects are inherent in the nature of the particular poison. For example, the molecule of staphylococcus toxin is relatively small. The small doses of this poison used by us appear to have increased the permeability of the glomeruli adequately to permit passage of the poison through the glomerular filter but not enough to produce appreciable amounts of albuminuria. Only 5 of our 12 dogs given this toxin showed albumin in the urine and these only from a trace to 2 plus. And yet the tubular epithelium in these dogs was sufficiently necrotic to indicate that the poison had passed through the glomerular walls with the filtrate. But even the largest molecule of a colloidal poison might ultimately injure the glomeruli sufficiently to permit it to escape into the filtrate and thus damage the tubular epithelium. Furthermore, a colloidal poison that had been sufficiently concentrated in the glomeruli by filtration to injure the endothelium would pass, in its newly concentrated form, with the blood into the peritubular capillaries, where it might be expected to produce further damage of these structures. Faber<sup>2</sup> has called attention to this possibility and has pointed out difficulties in the actual demonstration of the lesion.

4. The ideal colloidal nephrotoxic agent must produce lesions in the glomeruli that can be readily demonstrated and evaluated. The difficulty here lies in the marked differences of the structural patterns of the two parts of the renal units. The tubule is a relatively simple structure, consisting of cells arranged about a lumen. The glomerulus, on the other hand, is composed of about 50 long capillaries with their two ends

65. Babcock, C.: Anat. Rec. 71:233, 1938.

66. Ford, W. W.: Centralbl. f. Bakt. (Abt. 2) 58:129, 1913.

anchored at the hilus of the glomerulus and the remainder forming a mass of loops lying in the capsular space. These capillaries thus form a twisted skein in which it is next to impossible to differentiate separate loops. The changes which occur in them produce a mass effect rather than separate and distinct lesions that can be localized in individual cells and blood vessels. Thus it is far more difficult to visualize and describe definite pathologic changes in the glomeruli than in the tubules. It is therefore extremely difficult to standardize either the dose or the effects of a glomerular poison.

Certain shortcomings of an ideal colloidal nephrotoxic poison are inherent in its very nature. Its tendency to escape through injured glomeruli in the filtrate and to injure the tubular epithelium and the likelihood of its injuring the endothelium of the peritubular capillaries after being concentrated in the glomeruli have been mentioned. But a colloidal poison in the circulating blood differs from an inorganic, crystalloidal poison in an important respect. The inorganic nephrotoxic agent may combine with the proteins of the plasma, but it apparently leaves the blood stream only through certain excretory organs, of which the kidney is the most important. A colloidal poison, on the other hand, is rapidly removed from the blood by the reticuloendothelial system. This rapid change in the concentration of a colloidal poison in the blood stream renders the effects of such poisons uncertain and unpredictable. We attempted to obviate this difficulty by blockading the reticuloendothelial system of some dogs with india ink, but the results were not convincing.

#### SUMMARY

The experiments reported in this paper were based on the hypothesis that a dose of a colloidal poison could be found that when introduced into the circulating blood in amounts too small to injure other organs might be concentrated in the glomeruli through loss of water by filtration so as to injure these structures.

An ideal nephrotoxic colloidal poison should have the following characteristics: (*a*) its molecule should be large enough that it will not pass through the normal glomerular filter; (*b*) there must be a wide range between its minimum nephrotoxic dose and its minimum lethal dose; (*c*) it must not produce undesired side effects; (*d*) it must produce lesions in the glomeruli that can be readily demonstrated and evaluated.

In our experiments we used rattlesnake and moccasin venoms, the toxin of *Corynebacterium diphtheriae* and streptococcus and staphylococcus toxins. None of these proved to be an ideal colloidal nephrotoxic agent.

The venoms used in these experiments were furnished by Sharp & Dohme, Inc., and the bacterial toxins, by Parke, Davis & Company.

## PITUITARY LESIONS ACCOMPANYING OBESITY

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THE association of anatomic lesions of the pituitary gland and obesity has been noted in a number of conditions, such as Fröhlich's syndrome, Cushing's disease and tumors of the pituitary gland. Even with the rare types of obesity a variety of changes of this gland have been found. In Dercum's disease adenocarcinoma,<sup>1</sup> glioma,<sup>2</sup> adenoma or adenomatous hyperplasia,<sup>3</sup> and fibrosis<sup>4</sup> have all been described. Winkelmann and Eckel<sup>5</sup> found various pathologic changes in 8 of 11 pituitary glands which they examined. In Morgagni's syndrome adenomatous hyperplasia<sup>6</sup> and increase of the basophilic cells<sup>7</sup> have been reported. In Günther's syndrome<sup>7</sup> and in the Laurence-Moon-Biedl<sup>8</sup> syndrome increase of the basophilic cells has been found. Simmonds' disease due to postpartum necrosis of the pituitary gland has been observed associated with considerable gain in weight during the early stages,<sup>9</sup> but the same gain in weight is also found in an apparently normal postpartum course.<sup>9</sup> Trauma to the pituitary gland in man has been followed by obesity, according to Marañon (quoted by Echauz<sup>10</sup>). The evidence for this conclusion is purely circumstantial and dubious at best. The occurrence of obesity after experimental injury has been both advocated<sup>11</sup> and disputed.<sup>12</sup>

Of greater practical importance, perhaps, is the vast number of cases of obesity which do not fall into any of the aforementioned groups but are classified simply as cases of "constitutional" or "endocrine

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From the Laboratory of Pathology, City Hospital, Welfare Island.

1. Dercum, F. X., and McCarthy, D. J.: Am. J. M. Sc. **124**:994, 1902.
2. Burr, C. W.: J. Nerv. & Ment. Dis. **27**:519, 1900.
3. Price, G. E.: J. Nerv. & Ment. Dis. **36**:158, 1909. Foot, N. C.; Good, R. W., and Menard, M. C.: Am. J. Path. **2**:251, 1926.
4. Guillain, G., and Alquier, L.: Arch. de méd. expér. et d'anat. path. **18**: 680, 1906.
5. Winkelmann, N. W., and Eckel, K. L.: J. A. M. A. **85**:1935, 1925.
6. Henschen, F.: Hygieia **98**:65, 1936.
7. Ritter, W.: Frankfurt. Ztschr. f. Path. **52**:149, 1938.
8. Anderson, N. L.: J. Clin. Endocrinol. **1**:905, 1941.
9. Sheehan, H. L.: Quart. J. Med. **8**:277, 1939.
10. Echauz, F.: Arch. de med., cir. y especialid. **31**:606, 1929.
11. Biancardi, S.: Fisiol. e med. **2**:267, 1931.
12. Hetherington, A. W.: Endocrinology **26**:264, 1940.

obesity." Here, even in cases labeled "hypopituitary obesity" the diagnosis is based on the distribution of fat deposits, the characteristic metabolic pattern<sup>13</sup> and other clinical manifestations.<sup>14</sup> "Hypopituitary obesity" accompanied by retention of sodium and water has been ascribed by one writer<sup>14a</sup> to space-consuming intrasellar lesions—the "subclinical adenomata" of Costello.<sup>15</sup> All these concepts, however, are based almost exclusively on clinical evidence. Morphologic studies are relatively scanty, since obesity is rarely a fatal disease and is seldom seen at the autopsy table, particularly in persons who are in the early decades of life. Cases which have had a thorough metabolic study are even rarer.

The pathologic changes noted in the pituitary gland in "constitutional obesity" have been mainly changes in the proportions between the cell types, hyperplasias, benign tumors and stromal increase. Increase in the basophilic cells appears to be the most frequently observed feature; the range of incidence, however, is wide. Kraus<sup>16</sup> noted it in 81.8 per cent of his cases, Zeynek<sup>17</sup> in 81.3 per cent (26 of 32 cases), Bini<sup>18</sup> in 38.9 per cent (8 of 21 cases), Guizzetti<sup>19</sup> in 20 per cent (3 of 15), Kiyono<sup>20</sup> in 17 per cent (1 of 6) and Ritter<sup>7</sup> in a single case. Kraus expressed the opinion that this change was nonspecific since the identical finding is frequent in hypertension or renal disease. The eosinophilic cells were noted as increased in 1 case by Kiyono and decreased in 14 cases by Zeynek. The chromophobe cells have likewise been reported either as increased (Zeynek; Kiyono) or decreased (Zeynek). Adenomatous hyperplasia was found by Zeynek in 5 cases, by Guizzetti in 1 and by Kiyono in 1. Adenomas have been seen by all observers. Zeynek reported them in 6 cases, Guizzetti in 3 and Bini in 4. The adenomas have been of various types: chromophobe (Guizzetti; Bini), eosinophilic (Guizzetti), basophilic (Zeynek; Bini), "indifferent" (Bini) and choristoma (Bini).

Stromal increase has been noted by several observers. Zeynek found a "prominent increase" in 3 cases. Kup<sup>21</sup> noted "circumscribed fibrosis of the anterior lobe" of the pituitary gland of a 38 year old

13. Goldzieher, M. A.; Reimer, N. A., and Goldzieher, J. W.: Am. J. Digest. Dis. **12**:387, 1945.

14. (a) Goldzieher, M. A.: The Endocrine Glands, New York, D. Appleton-Century Company, Inc., 1939, p. 310. (b) Gottesman, A.: Med. Klin. **28**:15, 1932. (c) Lhermitte, V.; de Massary, V., and Trelles, J.'O.: Rev. neurol. **1**:375, 1934.

15. Costello, R. T.: Am. J. Path. **12**:205, 1936.

16. Kraus, J. E.: J. Clin. Endocrinol. **5**:42, 1945.

17. Zeynek, E.: Frankfurt. Ztschr. f. Path. **44**:387, 1933.

18. Bini, G.: Endocrinol. e pat. costit. **11**:168, 1936.

19. Guizzetti, F.: Arch. per le sc. med. **57**:325, 1933; **58**:1, 1934.

20. Kiyono, H.: Virchows Arch. f. path. Anat. **259**:388, 1926.

21. Kup, J.: Endokrinologie **6**:102, 1930.

woman. Kiyono noted a "sclerotic focus" in 1 case. Guizzetti described "senile fibrosis" in 7 and Bini "considerable fibrosis" in 5.

A number of other changes have been reported. In Kup's series of 31 pituitary glands 4 weighed over 1.0 Gm., 4 presented colloid cysts and 1 showed a chronic nonspecific granuloma. Marked increase in colloid was noted in isolated cases of extreme obesity by Kraus.<sup>22</sup> Evidence of gross compression was seen by Guizzetti in 3 cases; Bini found simple atrophy in 1, and Goldzieher<sup>14a</sup> observed degenerative changes and interstitial fibrosis with or without round cell infiltration to be frequent. Kiyono could find no relationship between obesity and metastatic lesions of the hypophysis in cases of carcinoma of the breast.

#### MATERIALS AND METHODS

The material consisted of the pituitary glands of 10 patients who came to autopsy at City Hospital, Welfare Island, New York, during the past five years. In each case the obesity was extreme; the distribution of fat was not described in sufficient detail for one to judge accurately whether it conformed to the various clinical types of "endocrine obesity." A few clinical and laboratory data were available in 2 instances only.

As control material 9 pituitary glands were used from persons who had no physical or historic evidence of past or present obesity, who had disease processes as similar as possible to those found among the obese, and who corresponded closely in age and sex.

The tissues were fixed in 10 per cent solution of formaldehyde, embedded in paraffin and stained with hematoxylin and eosin, the Heidenhain azocarmine modification of the Mallory connective tissue stain, a modified Mallory acid fuchsin stain, chromium-hematoxylin of Kraus<sup>23</sup> for acidophilic granules and cresofuchsin of Erdheim and Stumme<sup>24</sup> for basophilic granules. The steps of the modified Mallory acid fuchsin method are appended, as are those of the Kraus and Erdheim-Stumme methods, since descriptions of these methods, as far as we know, are not available in the American literature.

#### RESULTS

The frequency of changes in the ratio of the various cell types was highly suggestive. In all 10 of the obese persons there was an increase of the basophilic cells of the pituitary gland: This change was marked in 6 and moderate in 4. The eosinophils were decreased in 2. There was pronounced increase of the chromophobes in 5.

Small adenomas of the gland were found in 4 obese persons. In 2 they were chromophobe (patients 1 and 10 in table 1); in 2, basophilic (patients 5 and 6).

Senile fibrosis, shown by the prominence of the "core," was present in 3 obese persons (7, 8 and 10). On the other hand, a very peculiar,

22. Kraus, E. J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, p. 810.

23. Kraus, E. J.: Frankfurt. Ztschr. f. Path. 10:161, 1912.

24. Erdheim, J., and Stumme, F.: Virchows Arch. f. path. Anat. 46:1, 1909.

relatively circumscribed scarring, quite different from the senile changes, was found in 9 patients, being absent in only 1 (patient 2).

A marked increase in colloid of the pituitary gland was found in 3 obese persons; a moderate increase, in 1. Pronounced vacuolation of the basophilic cells was present in 6 of the obese persons; in 3 others this change was present to a slight extent.

The pertinent clinical and anatomic data on the obese persons are summarized in table 1 and the reports of 2 cases of obesity are given in detail.

CASE 1.—A 28 year old white woman entered the hospital because of multiple thrombophlebitis. The past history revealed a sudden gain in weight at the age

TABLE 1.—*Cases of Obesity*

Patient	Sex	Age	Pathologic Diagnosis	Body Proportions	Other Data
1	F	28	Thrombophlebitis migrans; rheumatic heart disease	Tremendously obese	Asthma; headaches; hyperorexia; hypertension
2	F	47	Cardiac hypertrophy; passive congestion; adenoma of adrenal cortex; hernia; bronchopneumonia	Extremely obese; breasts huge; abdominal fat 5 inches (12.5 cm.) thick	Asthma; cholecystitis; sporadic amenorrhea
3	F	46	Carcinoma of breast with metastases; slightly nodular adrenal gland	Short (5 ft. 3 in. [160 cm.]), very obese	Hirsuties of lip and chin
4	F	45	Thrombosis of internal carotid artery; cholelithiasis; fatty heart and liver	Obese	Chronic alcoholism some loss of weight
5	F	50	Choledocholithiasis; acute myocarditis; bronchopneumonia	Extremely obese, including abdomen, neck and legs	Menopause at 42
6	F	69	Adenoma of bronchus; bronchiectasis; cholelithiasis	Extremely obese; breasts and abdomen pendulous	
7	F	69	Atherosclerosis; cerebral hemorrhage	Extremely obese trunk; slender extremities	Hypertension
8	F	70	Bronchopneumonia	Extremely obese	Very mild diabetes
9	M	79?	Carcinoma of prostate; portal cirrhosis	Markedly obese	Very mild diabetes; rheumatoid arthritis
10	F	80?	Oholangiohepatitis; bronchopneumonia	Very obese, short	Blood sugar, 75; cholesterol, 182

of 17, which had progressed to extreme obesity. Asthma developed at 20 years. There were frequent headaches; the menses were irregular and scanty; the appetite was admittedly excessive. The blood pressure ranged from 180 systolic and 120 diastolic to 250 systolic and 120 diastolic. The fasting blood sugar was 114 mg. per hundred cubic centimeters. The blood cholesterol was not elevated. Death was due to multiple migrating thrombophlebitis causing infarcts of the lungs and the spleen. Autopsy revealed, in addition, severe cardiac hypertrophy and evidence of rheumatic heart disease. A complete report of the case has been published elsewhere.<sup>25</sup>

*Pituitary Gland.*—The pituitary gland did not appear enlarged. The microscopic observations were as follows: The capsule was thin. The acini were large and cellular. There was an irregular, marked increase of the stroma

25. Swirsky, M. Y., and Cassano, C. C.: J. Lab. & Clin. Med. 28:1812, 1943.

throughout with occasional frank scars, which were easily distinguished from the physiologic "core." Colloid was minimal. The eosinophils were numerous and of average size; they showed no changes of nucleus or cytoplasm. The basophils were definitely increased in number throughout the gland except perhaps at the posterior aspect, where eosinophils crowded them out. In the scarred areas basophils were present almost exclusively, and a suggestion of pseudopapillary formation was noted. A small proportion of the basophils showed vacuoles within the cytoplasm, but this was not a striking feature. The chromophobe cells seemed somewhat increased in number. There was a circumscribed adenoma of chromophobe cells just anterior to the intermediate zone; it measured 2.5 by 4.0 mm. in cross section. The surrounding tissue showed evidence of compression flattening, while the nodule itself manifested regressive changes.

CASE 2.—A 47 year old white woman entered the hospital because of cardiac failure. The past history revealed the following facts: Her weight at 16 years was 125 pounds (56.5 Kg.); at 20 years it was 150 pounds (68 Kg.); a marked gain began shortly after the birth of her first child. The weight at the time of admission was approximately 350 pounds (158.5 Kg.). There had been amenorrhea alternating with occasional menorrhagia for a long time; asthma was present; cholelithiasis had necessitated removal of the gallbladder. The blood pressure ranged about 260 systolic and 140 diastolic. The fasting blood sugar was 110 mg., the uric acid 3.3 mg. and the cholesterol 125 mg. per hundred cubic centimeters. The roentgenographic appearance of the sella turcica was said to be normal. Death was due to intractable cardiac failure and bronchopneumonia. Autopsy revealed, in addition to general passive congestion, marked hypertrophy of the right and left sides of the heart, a small adenoma of the cortex of the adrenal gland and an incarcerated hernia.

*Pituitary Gland.*—The capsule was thin. The gland appeared rather cellular, especially as the cells generally were rather small and the acini not always distinct. The stroma was not at all prominent save for a single extension of the core far into the anterior lobe. Colloid was minimal. The eosinophils were present in fair numbers, and no changes of nucleus or cytoplasm were seen. The basophils were markedly increased in patchy fashion, forming definite nests of cells. Vacuoles were seldom large and were found only in a small proportion of the cells. The chromophobes were definitely increased in number, and this change was most prominent in those areas showing a predominance of basophils.

The observations in the 2 cases just described serve to illustrate the fact that the changes observed are by no means predictable. The history of case 1 is not entirely typical of "hypopituitary obesity," whereas that of case 2 is far more suggestive. The results of the laboratory studies which were made do not conform to the metabolic pattern of "hypopituitary obesity" in either case. Yet rather pronounced changes were seen in case 1, whereas increases of the basophils and chromophobes were the only essential findings in case 2.

*Controls.*—In the 9 control pituitary glands a slight increase of the basophils was found in a single gland; no increase was seen in the remainder. No noteworthy changes of the eosinophils or of the chromophobes were found in any of the controls. No adenomas were found. Increased colloid was noted in 5; this was marked in 3 and moderate in 2. Vacuolation of the basophilic cells was marked in 5 glands and slight in 1. The pertinent clinical and anatomic data on the controls are summarized in table 2.

## COMMENT

The most interesting feature found in this series was the stromal change which appeared to be so characteristic. The connective tissue of the hypophyses which I have examined falls into three distinct categories. There is the physiologic stroma—the so-called "core"—which is seen in the region between the anterior and the intermediate lobe and which from there sends extensions forward. The connective tissue is of the characteristic hyaline variety with typical cigar-shaped nuclei and thick, wavy bundles of collagenous fibers (fig. 1). In addition, blood vessels and lymphatics accompany these strands and thereby make their identification easier. It is my impression that the connective tissue of the core increases, or at least becomes more prominent, with age; the essential characteristics, however, do not change.

TABLE 2.—*Controls*

Patient	Sex	Age	Pathologic Diagnosis	Body Proportions	Other Data
1	F	49	Hepatic cirrhosis; ruptured esophageal varices	Average.	
2	F	49	Pyelonephritis; multiple sclerosis	Average	Blood chemically normal
3	F	60	Cirrhosis; esophageal varices	Somewhat emaciated	Chronic alcoholism
4	F	62	Pulmonary embolism; calcified colloid goiter	Average	
5	F	60+	Miliary tuberculosis; cholelithiasis	Emaciated	
6	F	65	Radical mastectomy; coronary arteriosclerosis	Emaciated	Hypertension; old hemiplegia
7	F	68	Cardiac thrombus; emboli	Average	
8	M	74	Paget's disease; cholelithiasis	Average	
9	F	78	Bronchopneumonia	Emaciated	Mild diabetes

In the process of aging the interacinous stroma also becomes more prominent. This tissue shares many of the characteristics of the core, having a tendency to run in strands and showing numerous, occasionally somewhat vesicular nuclei. The fibers are rather finer than those of the core, although there is still a tendency to group into bundles (fig. 2). In all the controls in which an increase of stroma was noted, the change falls into one of the aforementioned groups.

On the other hand, 9 of the glands of the obese patients showed a strikingly different change. Here were observed localized, prominent increases of the connective tissue suggestive of small scars (fig. 3). The stroma in these areas was quite different from that described in the foregoing paragraph. The collagenous tissue contained but few nuclei, its fibrils were delicate and nonhyaline, and the adjacent parenchyma showed changes as well (fig. 4). Here the acini tended to be atrophic except where they were replaced by areas of almost adenomatous

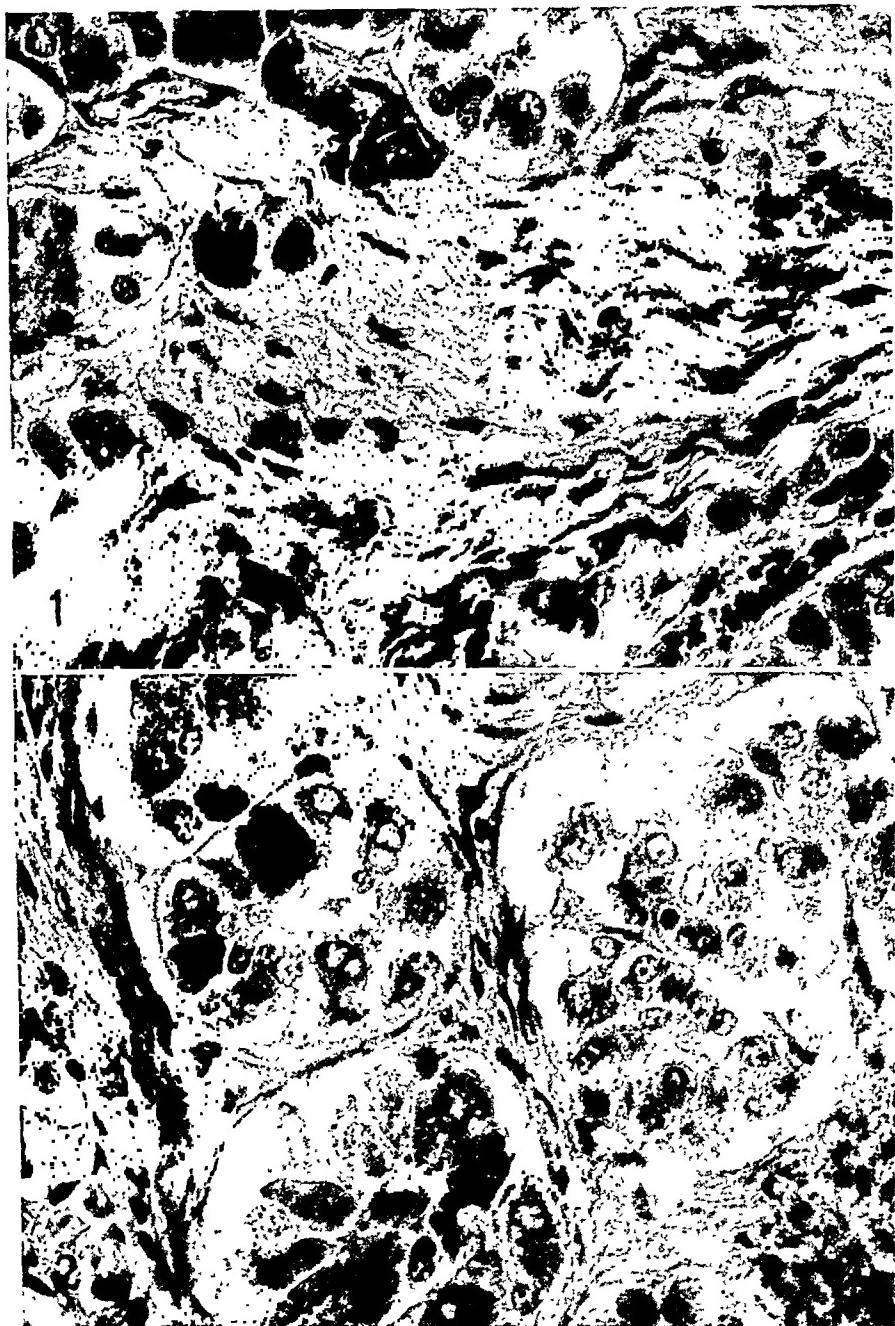


Fig. 1.—Section of the physiologic "core" of the pituitary gland, showing the wavy bundles of hyaline connective tissue, the numerous typical connective tissue cell nuclei and a blood vessel. Modified Mallory acid fuchsin connective tissue stain.  $\times 450$ .

Fig. 2.—Senile fibrosis. Note the amply nucleated bundles of slightly wavy collagenous fibers and the large acini containing a variety of healthy-looking cells. Modified Mallory acid fuchsin connective tissue stain.  $\times 450$ .

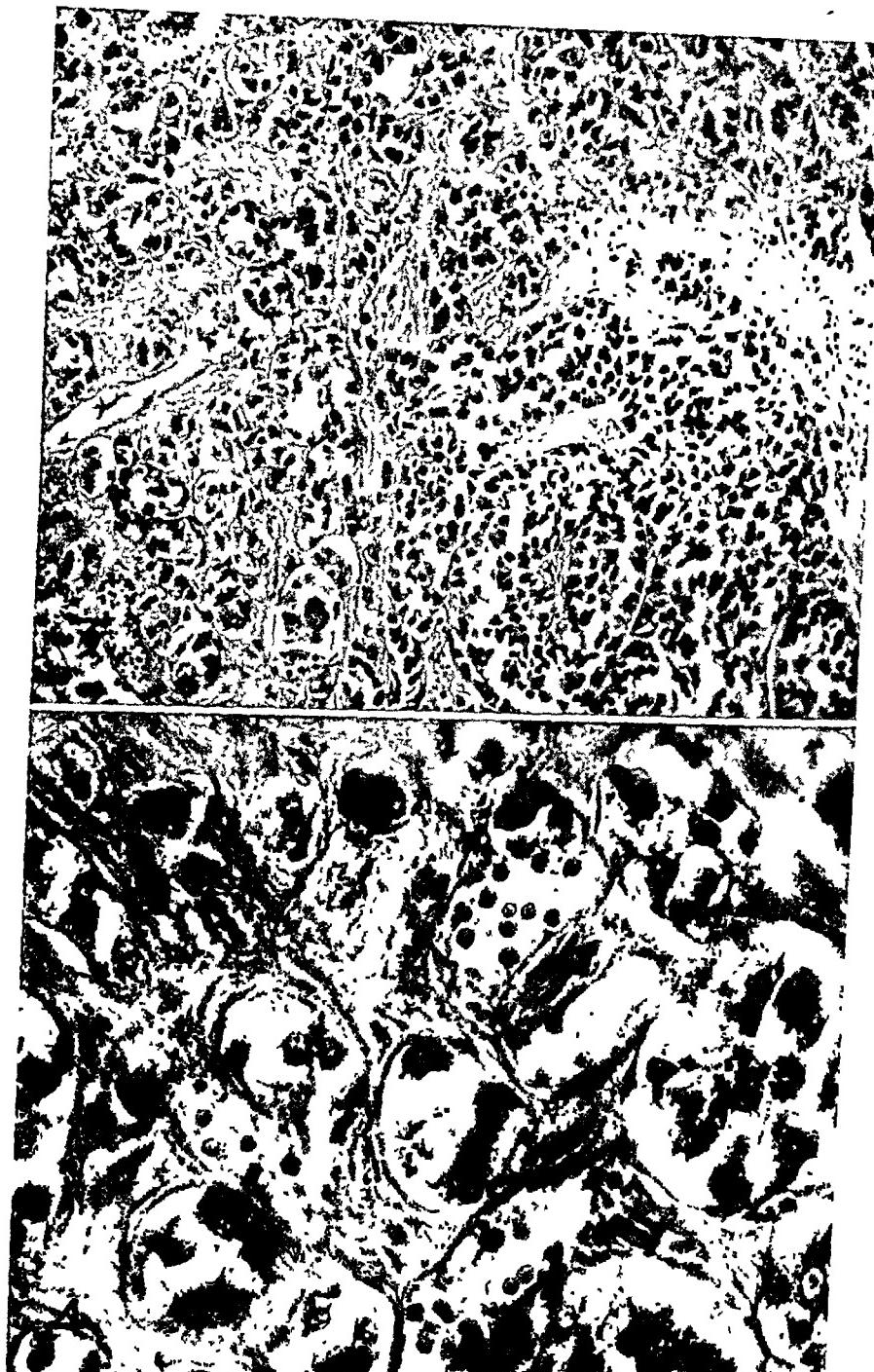


Fig. 3.—Circumscribed area of diffuse interstitial fibrosis with atrophic acini (at left) giving way to markedly hyperplastic areas (at lower right). Modified Mallory acid fuchsin connective tissue stain.  $\times 140$ .

Fig. 4.—High power view of a part of the section shown in figure 3. Note the finely fibrillar character of the scar tissue, the absence of nuclei or of hyaline appearance. The acini are atrophic and consist exclusively of basophilic cells. Moderate congestion is evident. Modified Mallory acid fuchsin connective tissue stain.  $\times 450$ .

atous hyperplasia. The cells in these scarred areas were decreased in size and nearly always basophilic in character. In some hyperplastic areas the proliferation assumed almost pseudopapillary proportions.

The presence of focal scarring has been described by a number of the authors cited; most of them dismiss the change as senile atrophy. It is possible that no distinction was made between true senile fibrosis and the morphologically quite different areas of scarring which I observed only in the pituitary glands of obese persons and never in the controls falling into the "senile" age group. Moreover, advanced parenchymal changes in the vicinity of these peculiar scars suggest that there may have been qualitative as well as quantitative change in the local secretory activity.

The present material shows the predominance of basophil cells noted by others. It does not, however, suggest that there is any correlation between obesity and the accumulation of colloid, as this change was noted in equal proportions of the glands of obese persons and controls. The vacuolation of the basophilic cells also does not differ markedly in the two groups.

An incidence of adenoma in 40 per cent in the group of obese persons as against none in the controls is entirely within the range of statistical variation in such small samples and hence is not significant. However, in adding together this material and that of Bini, Guizzetti and Zeynek I find an incidence of 23 per cent (18 of 78 cases). This is almost identical with the figure of Costello—22.5 per cent—arrived at from a study of a thousand routinely received pituitary glands. Although it would be valuable to have a statistical breakdown of Costello's data as regards the presence or the absence of obesity, the similarity of his figure to the incidence I have just calculated apparently does not support the hypothetic role of the subclinical adenoma in the causation of obesity.

The interpretation of these findings and their relation to obesity and the metabolic status generally must be approached with great caution. There is no evidence to prove that the scarring as an indication of pituitary damage is causally related to obesity. On the other hand, one should not fall prey to the post hoc fallacy and assume that these changes are the consequence of the metabolic disturbances. With the available data, the association of these two facts can merely be noted; no conclusions may be drawn.

It is obvious that the material studied under the generic term "constitutional" or "endocrine obesity" is a heterogeneous group, and consequently a uniform and characteristic appearance of the pituitary glands is hardly to be expected. Moreover, it must be conceded that functional alterations of the pituitary gland not detectable by present day histologic technics are entirely possible. The final answer to this problem will rest in the accumulation of cases in which the results of

careful histologic study of the pituitary gland can be correlated with the clinical history, the distribution of fat and the metabolic pattern.

#### SUMMARY

In a series of 10 cases of "constitutional obesity" the pituitary changes consisted of a peculiar scarring of the stroma and a predominance of the basophils with some increase in the chromophobes. No such changes were seen in 9 selected controls. Although this feature of the pituitary glands of obese persons was striking, the question of its causal relation to the development of obesity must remain in abeyance. The term "constitutional" or "endocrine obesity" appears to comprise a heterogeneous group of cases.

#### APPENDIX

1. *Modified Mallory Acid Fuchsin Connective Tissue Stain:*

1. Deparaffinize and stain with Delafield's hematoxylin ten to fifteen minutes, followed by acid alcohol and then by weak ammonia water.
2. Stain with 0.1 per cent acid fuchsin for thirty seconds to one minute.
3. Stain in Mallory's aniline blue-orange G-phosphomolybdic acid ten to twenty minutes.
4. Dehydrate and clear in ascending alcohols and xylene then mount in balsam.

2. *Kraus's Chromium-Hematoxylin Stain for Acidophilic Granules:*

1. Deparaffinize and mordant overnight in 5 per cent aqueous potassium dichromate.
2. Stain in Kultschitzky hematoxylin for twenty-four hours. (This is prepared by dissolving 2.0 Gm. of hematoxylin in sufficient dehydrated alcohol, then making the solution up to a volume of 100 cc. with 2 per cent acetic acid. Ripening is necessary.)
3. Wash in water briefly.
4. Differentiate for fifteen minutes to two hours in a 1:1 dilution of Weigert's differentiator (borax 2.0 Gm., potassium ferricyanide 2.5 Gm., water 100 cc.).
5. Counterstain with picrofuchsin if desired.

3. *Erdheim-Slumme Cresofuchsin Stain for Basophilic Granules:*

1. Deparaffinize. Overstain heavily with Orth's lithium carmine<sup>26</sup> and wash in water.
  2. Stain for twenty-four hours in a closed container of cresofuchsin solution (cresofuchsin 0.3 Gm., concentrated hydrochloric acid 2 cc., 70 per cent ethyl alcohol 100 cc.).
  3. Wash in 70 per cent alcohol, clear in carbolxylene and xylene and mount in balsam.
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26. If counterstaining is not desired, Orth's carmine may be eliminated.

# Case Reports

## ADENOACANTHOMA OF THE STOMACH

GEORGE STRASSMANN, M.D., WALTHAM, MASS.

HETEROLOGOUS adenoacanthoma (Pasternack<sup>1</sup>), or adenocarcinoid (Herxheimer<sup>2</sup>), a mixed glandular and squamous cell carcinoma of the stomach, is rare. There is variance of opinion in explaining the occurrence of tumors of this type, which arise in areas where normally no squamous cell epithelium is present. In a recent review Wood<sup>3</sup> collected 10 cases from the literature and added 2 of his own. The fact that in only 2 of the 12 cases the tumor was situated in an extrapyloric region at some distance from the cardia (Martin and Pollossen<sup>4</sup>; Scheffler and Falk<sup>5</sup>) indicates that adenoacanthoma has a predilection for the pyloric region. In 1 instance adenoacanthoma extended from the pylorus to the cardia (Takagi<sup>6</sup>). The case now reported is the second instance of adenoacanthoma extending beyond the pyloric area to the cardia.

### REPORT OF A CASE

An 85 year old white man was admitted to the Metropolitan State Hospital, March 29, 1944, with a diagnosis of senile psychosis (paranoid type). In the last few months he had become restless, confused and assaultive, with persecutory delusions. His mental status did not change during his stay in the hospital. The medical history was not significant except for an operation on the prostate ten years before. On admission he was poorly nourished and had slight hypertension and general arteriosclerosis.

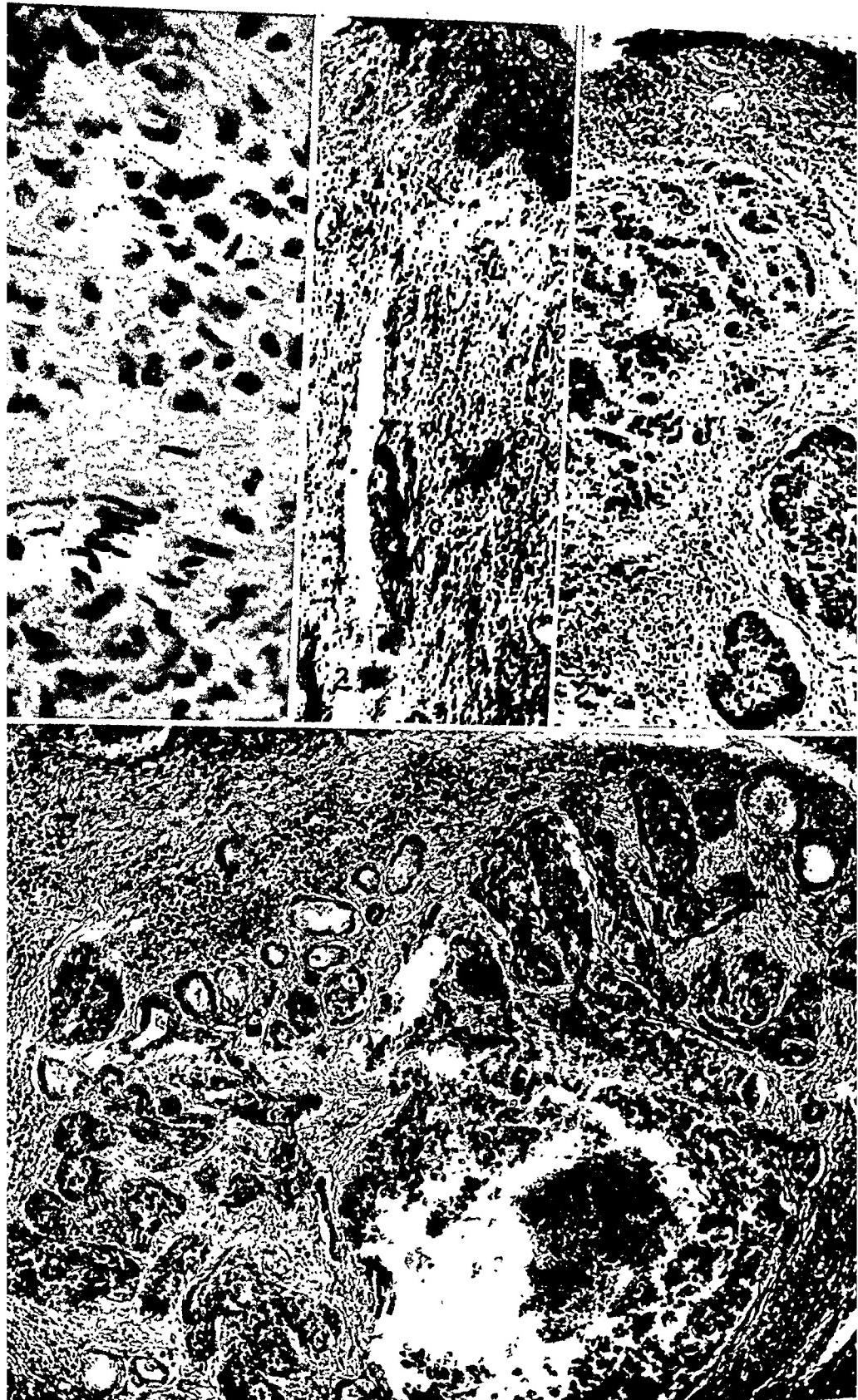
The hemoglobin content was 94 per cent (Sahli); the red blood cell count was 4,830,000 and the white cell count 6,600; the Hinton test was negative. In December 1944, during an infection of the respiratory tract, the hemoglobin content was 70 per cent (Sahli) and the red blood cell count 3,800,000. Soon afterward he refused to take food, although he expressed no specific complaints. He started to lose weight and died from a terminal infection of the respiratory tract, Feb. 18, 1945.

*Autopsy* (nineteen hours after death).—The cause of death was confluent bronchopneumonia of both lungs with fibrinous pleuritis. Cerebral and general arteriosclerosis, nephrosclerosis, and hemorrhagic cystitis were also present. Unusual and unexpected were the findings in the stomach. This organ was

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From the Laboratory of the Metropolitan State Hospital.

1. Pasternack, J.: Am. J. Path. **11**:541, 1935.
2. Herxheimer, G.: Beitr. z. path. Anat. u. z. allg. Path. **41**:348, 1907.
3. Wood, D.: Arch. Path. **36**:177, 1943.
4. Martin, J. F., and Pollossen: J. de méd. de Lyon **17**:553, 1936.
5. Scheffler, M. M., and Falk, A. B.: Am. J. Cancer **38**:359, 1940.
6. Takagi, C.: Gann **31**:137, 1937; cited by Wood.<sup>3</sup>



(See legend on opposite page)

empty. The wall was thickened. From the mucosal surface numerous small grayish nodules protruded into the lumen. The thickening of the wall and the nodules extended from the pyloric region through the fundus and both curvatures to the cardia. The thickening of the wall was irregular. Between the cardia and the pylorus there were several areas in which the wall was normal in thickness and appearance. On the cut surface the walls showed two different layers, well demarcated from each other. The inner layer was thicker. It was white and firm and consisted of hypertrophic mucosa and submucosa. The outer layer was grayish red and consisted of the muscular tunics and the serosa. In most places the entire wall measured from 6 to 8 mm. while the inner layer was from 3 to 5 mm. thick. The wall in the prepyloric region was the thickest. Here it measured 11 mm., while the inner layer was 7 mm. thick. A few enlarged, firm, grayish periportal lymph nodes and two small nodules in the liver proved the neoplastic nature of the process. The cardia, the lower part of the esophagus and the small and the large intestine were grossly normal. There were no metastases elsewhere.

*Microscopic Examination.*—Sections from different thickened areas of the gastric wall gave essentially the same picture. Normal mucous glands were occasionally seen, but in most of the areas the mucosa was replaced either by accumulations of round cells or by long columns of pale polymorphous large cells with eccentric nuclei. The size and the shape of these pale cells varied to a considerable degree. The mucosa was separated from the submucosa by muscularis mucosae. The submucosa consisted of a thick layer of connective tissue fibers with many blood vessels and scattered accumulations of round and plasma cells. The lymphatic spaces were infiltrated by columns of the same large polymorphous pale cells with eccentric nuclei, which showed a glandular and alveolar arrangement in some places. In other areas, such cells had an anaplastic appearance. Similar cells infiltrated the spaces between the muscle bundles and the perineural spaces. Both muscle layers were hypertrophic and contained an increased amount of connective tissue. The tumor cells showed mitotic figures in different areas (fig. 1). The area of the greater curvature 3 cm. distant from the cardia was the only one where a quite different picture of the tumor was observed. Grossly, the thickened area here looked similar to other thickened areas. Microscopically, however, there was a sudden transition at the cardiac edge of the tumor from normal glandular mucosa to a mucosa formed by solid layers of large hyperchromatic polygonal cells with large dark nuclei. Some of the cells were flat, others were elongated with oblong nuclei, and others were almost round with round nuclei. The cells and their nuclei

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#### EXPLANATION OF PLATE.

Fig. 1.—Infiltration of the submucosa by polymorphous tumor cells with eccentric nuclei, accompanied by round cells. Hematoxylin and eosin;  $\times 391$ .

Fig. 2.—Mucosa covered by squamous epithelial cells. Fibroplastic proliferation is seen in the submucosa. Hematoxylin and eosin;  $\times 187$ .

Fig. 3.—Section from the same area as figure 2. Squamous cell carcinomatous infiltration of the submucosa. Some of the tumor cells have a basal cell character. Hematoxylin and eosin;  $\times 188$ .

Fig. 4.—Same area of the submucosa as that in figures 2 and 3. Displaced mucous glands with adenocarcinoma may be seen. Hematoxylin and eosin;  $\times 188$ .

varied in size and shape. Some showed a whorl-like arrangement but no glandular structures. Cell masses invaded the submucosa and the submucosal vessels. Their appearance in some places was that of basal cell carcinoma, although most of the cells presented the picture of squamous cell carcinoma with cornified epithelial pearls and keratinizations (fig. 3). Accumulations of round cells replaced parts of the mucosa and extended between the tumor masses into the hypertrophic submucosa, which consisted of a thick layer of connective tissue fibers. A small area of the mucosa was covered by normal squamous epithelial cells, which in one region had changed their character. The cells there became more irregular and hyperchromatic and invaded the submucosa in solid masses (figs. 2 and 3). In the submucosa of this area displaced mucous glands were lined by cuboidal epithelial cells, their lumens were distended and they were surrounded by clusters of round cells. Some of the glandular cells had proliferated and formed several layers of hyperchromatic cells with dark nuclei. The lumen and the glandular structure were still visible. However, the picture was definitely that of adenocarcinoma (fig. 4). In other glands, the lumen was obliterated, and the glandular structure had disappeared and was replaced by solid layers of hyperchromatic, polygonal cells with large nuclei. Cornified epithelial pearls were seen similar to those observed in the tumor of the mucosa of this region.

The cardia and the few areas of the gastric wall which grossly appeared normal showed microscopically normal glandular mucosa, submucosa and muscularis. There were some accumulations of round cells between the mucosal glands and in the submucosa.

Only a small amount of lymphoid tissue was visible at the edge of an involved periportal lymph node. Most of the node consisted of solid layers of large irregular hyperchromatic cells with large dark nuclei. These cells varied in size and shape; some were rounded, some were elongated and some were polygonal and irregularly shaped. The center of the node was largely necrotic. A number of mitotic figures and many cornified epithelial pearls were seen. The solid layers of tumor cells alternated with bundles of connective tissue fibers containing clusters of round cells.

The liver cells adjacent to a tumor node contained many fat droplets and brownish pigmented granules. The tumor nodules consisted of solid layers of polygonal hyperchromatic cells of varying size and shape with large dark nuclei. Mitotic figures and many cornified epithelial pearls were seen. Connective tissue fibers were observed between the layers of tumor cells; some of the tumor cells showed a whorl-like arrangement. The network of connective tissue contained accumulations of round cells and histiocytes with hemosiderin.

Nothing remarkable was observed in other organs except arteriosclerotic changes.

#### COMMENT

Several explanations have been given as to the cause of heterologous adenoacanthoma, or adenocancroid, of the stomach. According to Herxheimer,<sup>2</sup> the mixed glandular and squamous carcinomatous cells were derived from undifferentiated embryonic cell groups in the gastric mucosa rather than from displaced esophageal cells. Krompecher,<sup>7</sup> on the other hand, expressed the belief that nonembryonic undiffer-

7. Krompecher, E.: Beitr. z. path. Anat. u. z. allg. Path. 72:163, 1924.

entiated basal cells, normally present in the gastric mucosa, developed into the cells of squamous and glandular carcinoma. Following this theory, Wood<sup>3</sup> expressed the opinion that the carcinogenic agent, whatever it was, stimulated these basal cells to proliferate along two different lines leading to squamous and glandular cells. The possibility of true metaplasia of normal mucosal cells under certain conditions preceding the neoplastic proliferation or occurring at the same time was emphasized by Lubarsch.<sup>8</sup> Oberling<sup>9</sup> and associates explained adenoacanthoma of the stomach as a metaplasia of adult cells in 1 instance and as congenital heterotopy in another, reported by Oberling later.

The attempts to produce squamous cell metaplasia of gastric mucosa in animals were only occasionally successful (Fuetterer<sup>10</sup>; Klein and Palmer<sup>11</sup>; Howes and Vivier<sup>12</sup>). Recently, however, Stewart and Lorenz<sup>13</sup> succeeded in producing adenocarcinoma and adenoacanthoma in the forestomach and in the pyloric stomach of mice with injections of methylcholanthrene. They concluded from their experiments that under carcinogenic influences a normal glandular mucosa could differentiate into glandular and squamous cell neoplastic elements.

The case of adenoacanthoma reported in this paper is different from those described in the literature.<sup>14</sup> The tumor had not been suspected during life, although at autopsy it was found that large areas of the gastric wall from the pylorus to the cardia were involved in a neoplastic process preceded or accompanied by chronic hypertrophic gastritis. The tumor was predominantly a more or less anaplastic type of adenocarcinoma, infiltrating all layers of the wall. The adenocarcinomatous cells were of an irregular pale polymorphous type with eccentric nuclei. The part of the tumor which consisted of both adenoacanthomatous and squamous cell elements was limited to a small circumscribed area of the greater curvature near the cardia, covered by squamous epithelium similar to that normally present in the esophagus. There was no continuation of the squamous epithelium from this region to the cardia. There are two possibilities to explain the presence of this squamous epithelium. It could represent congenital heterotopy, a displacement

8. Lubarsch, O.: Verhandl. d. deutsch. path. Gesellsch. (1906), 1907, p. 198.

9. Oberling, C., and Wolf, M.: Bull. Assoc. franç. p. l'étude du cancer **16**:68, 1927. de Martel, T.; Oberling, C., and Pernet, J.: ibid. **19**:470, 1930.

10. Fuetterer, G.: Ergebn. d. alg. Path. u. path. Anat. **9**:706, 1903.

11. Klein, A. J., and Palmer, W L: Arch. Path. **29**:814, 1940.

12. Howes, E. L., and Vivier, P. J.: Am. J. Path. **12**:689, 1936. .

13. Stewart, M. L., and Lorenz, E. J.: J. Nat. Cancer Inst. **2**:193, 1941; **3**: 175, 1942.

14. Bödecker, F.: Ztschr. f. Krebsforsch. **24**:406, 1927. Pasternack,<sup>1</sup> Herxheimer,<sup>2</sup> Wood,<sup>3</sup> Martin and Pollossen.<sup>4</sup> Scheffler and Falk.<sup>5</sup> Takagi.<sup>6</sup> Lubarsch.<sup>8</sup> Oberling and others.<sup>9</sup>

of esophageal epithelium. It seems more likely, however, that the squamous epithelium was derived from undifferentiated basal cells in the gastric mucosa which had undergone metaplastic change after a chronic inflammation of the stomach. The patient's age (86 years) and the presence of chronic hypertrophic gastritis make the latter conclusion more probable. Some of the squamous cells had become neoplastic and formed a squamous cell carcinoma with cornified epithelial pearls invading the submucosa. Some parts of the tumor in this region had the appearance of basal cell carcinoma and originated probably from undifferentiated basal cells. Epithelial cells of displaced mucous glands in the hypertrophic submucosal layer of the same region had proliferated into a mixed adenoacanthoma with glandular and squamous cell carcinomatous structures. Such displaced mucous glands associated with gastric carcinoma have been found not infrequently in the stomachs of elderly people (Lubarsch<sup>8</sup>). It is interesting to note that the metastases of the liver and the periportal lymph nodes showed pure squamous cell carcinoma.

It is difficult to decide in which area of the stomach the neoplastic process started. However, it can be stated that the carcinogenic agent, whatever it was, had stimulated epithelial cells of the gastric mucosa and of mucous glands to proliferate along three different lines. First, the glandular mucosa of large parts of the stomach from the pylorus to the cardia had developed into diffuse adenocarcinoma invading all layers of the stomach wall. Second, metaplastic or heterotopic squamous epithelium covering a small area of the stomach near the cardia had proliferated into squamous cell carcinoma. Because of the admixture of basal cell carcinomatous elements, undifferentiated basal cells probably participated in the formation of this part of the tumor. Third, epithelial cells lining displaced mucous glands in the submucosa of the same region had formed an adenoacanthoma with mixed glandular and squamous cell carcinomatous elements.

Heterotopic or metaplastic squamous epithelium covering the gastric mucosa in association with adenoacanthoma has not been reported in any other case of adenoacanthoma of the stomach; in all other cases noncancerous squamous cell metaplasia was consistently absent (Wood<sup>3</sup>).

#### SUMMARY

Adenoacanthoma of the stomach was observed associated with chronic hypertrophic gastritis at autopsy of a man 86 years old. It was predominantly more or less anaplastic and extended from the pyloric region to the cardia. The squamous cell carcinomatous and adenoacanthomatous element was limited to a small area of the greater curvature near the cardia covered by metaplastic or heterotopic squamous epithelium.

This epithelium had proliferated into squamous cell carcinoma with admixture of basal cell elements deriving from undifferentiated basal cells. A mixed type of adenoacanthoma had been formed by epithelial cells lining displaced mucous glands in the same region. Different epithelial elements, glandular mucosal cells, basal and squamous cells had been stimulated under a carcinogenic influence and had differentiated into mixed carcinomatous elements. The metastases in the liver and in regional lymph nodes showed pure squamous cell carcinoma with many cornified pearls.

## Notes and News

**Appointments.**—George M. Hass, chief of pathology in the School of Aviation Medicine, Randolph Field, Texas, has been appointed professor of pathology in the Rush-Presbyterian Hospital Division of the University of Illinois College of Medicine.

D. A. Mactadyen, chief of the division of chemistry and physics at the Army Medical Center, Washington, D. C., has been appointed professor of biochemistry in the Rush-Presbyterian Hospital Division of the University of Illinois.

Robert E. Stowell, assistant professor of pathology in the Washington University School of Medicine, St. Louis, has been awarded a fellowship by the Commonwealth Fund of New York to enable him to study in Sweden under Dr. Torbjorn Caspersson, chief of the Swedish Institute for Cytologic Research.

Lieutenant Colonel Elson B. Helwig, who has been in the Army of the United States for the past three years, has returned to the department of pathology of Washington University School of Medicine.

**Deaths.**—Mazyck Porcher Ravenel, first bacteriologist of the Pennsylvania State Livestock Board, died January 14, aged 84 years.

**Awards.**—Brigadier General Stanhope Bayne-Jones, deputy chief of the Preventive Medicine Service, Office of the Surgeon General, was recently awarded the Distinguished Service Medal for his outstanding "contribution to the maintenance of health within the Army. As administrator of the Epidemiological Board, he directed the extension, administration and military application of the worldwide research and control program conducted by this board and its ten commissions." Before taking up his military duties in 1942 General Bayne-Jones was professor of bacteriology at Yale University.

The John Scott Award of the Philadelphia Board of City Trusts has been given to Ernest W. Goodpasture, professor of pathology and dean of the Vanderbilt University School of Medicine, Nashville, Tenn., for his "development of a method for the cultivation of viruses that have made hitherto unknown vaccines possible." Edwin J. Cohn, professor of biochemistry, Harvard Medical School, Boston, received a similar award for developing "a substitute for dried blood plasma used in the treatment of shock in the war."

**Society News.**—The Society of American Bacteriologists announces the election of the following officers to serve in 1946: James Craigie, Toronto, Canada, president; Thomas Francis Jr., Ann Arbor, Mich., vice president; Leland W. Parr, Washington, D. C., secretary-treasurer; M. J. Rosenau, Chapel Hill, N. C., and Frederick Smith, Montreal, Canada, councilors.

**Friends of Medical Research.**—This group has been organized under the auspices of the New York Academy of Medicine to inform the public about animal experimentation and what such experimentation has contributed to science and health. Simon Flexner has been named honorary president. The first efforts are directed toward the defeat of the DiCostanzo-Davidson bill in the legislature of New York, which proposes to make research work with dogs unlawful.

## Book Reviews

**Pulmonary Tuberculosis in the Adult: Its Fundamental Aspects.** By Max Pinner, M.D., chief of the division of pulmonary diseases, Montefiore Hospital for Chronic Diseases, New York; clinical professor of medicine, College of Physicians and Surgeons, Columbia University, New York. Price, \$7.50. Pp. 579, with 59 illustrations. Springfield, Ill.: Charles C Thomas, Publisher, 1945.

In this extremely well written and thoughtful book Dr. Pinner presents the rich fruits of his long experience with tuberculosis as a clinician and as a devoted and constantly inquiring student of the disease. It is not his purpose in this book to give directions for diagnosis and treatment but rather to present a synthesis of the multiple factors that bear on phthisiogenesis, in order to provide a basis for the understanding of the disease and a foundation for practical work. The result is a treatise that cannot fail to be of the greatest interest and importance to all who are concerned with the problem of pulmonary tuberculosis in the adult. The clinical aspects are closely and admirably correlated with the underlying pathologic alterations. Few clinical students of tuberculosis have as sound and as clear an understanding of the pathology of the disease as has Dr. Pinner, and his clinico-pathologic correlations are masterful. No less masterful are the chapters that outline the principles bearing on incipient tuberculosis and on collapse therapy. While it is not to be expected that all of any person's views on controversial matters pertaining to therapy will be shared by every one who deals with tuberculosis, still there speaks here a deeply thoughtful and keenly observant clinical student of the disease, from the vantage point of an abundant and well used experience.

While the portions of the book dealing with the clinical and the morphologic aspects of tuberculosis, with which Dr. Pinner is thoroughly experienced at first hand, are superb, in the portions devoted to fundamental immunologic principles and to the interpretation of experimental studies the author treads less familiar ground, and the treatment is correspondingly less ably critical, less logical and too often colored by bias and by unwarranted dogmatism. In his preface he states that his guiding principle in the writing of this book was to achieve consistency in all of its aspects, in order to present a plausible picture, rather than to present the available evidence impartially, that he therefore expects the criticism of personal bias and that he regards that as a small price to pay for coherence and clarity. The wisdom of that procrustean attitude in dealing with matters of science must be questioned. At all events, in view of it and of the fact that statements supporting his own views are often presented with a dogmatic finality that is contrary to the existing evidence, it will be well to bear his warning preface constantly in mind. In his discussion of basic immunologic principles, the presentation of pertinent evidence, which often exists even in the papers quoted, appears to be sacrificed in the interests of maintaining the consistency of his own views. Since it would be altogether unfair for the reviewer to make these statements without documenting them, the following examples have been selected:

On page 111, in support of the view that resistance is determined mainly by the degree of local inflammatory reaction, Dr. Pinner quotes a study on animals with different degrees of native resistance as demonstrating that "invariably, the resistant animals showed the quickest response" to a local, intracutaneous injection of killed tubercle bacilli. Column I of table 4 in the article referred to shows that this was not the case. There was no difference between the reactions of normal animals of the most resistant and of the most susceptible groups, nor is it claimed in the paper that there was.

On page 104 he cites the work of one investigator as evidence that in experimental reinfection the tubercle bacilli are destroyed without any preliminary multiplication. He fails to mention that this was not confirmed by subsequent equally competent investigators.

On page 107 he states that it has been "unequivocally shown" that the phagocytosis of tubercle bacilli by macrophages is not enhanced by immune serum. The

reverse, however, has long been well established, and the reviewer knows of no documented studies to the contrary.

In the same discussion it is stated as an "incontestable fact" that passive transfer of acquired resistance in tuberculosis "is impossible." It is not pointed out that no adequately controlled study of the matter has yet been carried out, nor that in the better controlled of the existing studies evidence of passive transfer of resistance was obtained.

On page 94 it is stated that when a massive dose of nonvirulent bacilli is injected subcutaneously an abscess develops, there is limitation of spread of the bacilli and healing occurs. The limitation of spread and the suppression of the bacilli are attributed to the acute inflammation. The post hoc logical fallacy is obvious; and the author does not mention the fact that a similar injection of virulent bacilli will likewise cause an abscess but that in this case widespread devastating disease results. The outcome in the case of the nonvirulent bacilli is clearly a result of the nature of the bacillus and not of the inflammatory reaction.

On page 98 it is stated as an "incontrovertible fact" that tuberculous tissue is required for the acquisition of resistance in tuberculosis. It is true that up to the present resistance has been acquired only on the introduction of entire bacilli into the body and that the complex bacillus causes the development of tuberculous tissue. Here again, however, the author falls into a post hoc logical fallacy by concluding that the tuberculous tissue itself is necessary for the acquisition of resistance. In the present state of information it would certainly be a great mistake to regard that as an "incontrovertible fact." Highly potent antigens that immunize in the absence of inflammatory tissue reactions have been isolated from the pneumococcus and from other bacteria, and there is good reason to believe that the same end may be accomplished in the case of the tubercle bacillus.

On page 118 the author states dogmatically that it has never been demonstrated that anything more than cutaneous reactivity can be abolished by desensitization. He does not mention the thoroughly controlled studies of the numerous investigators who have demonstrated clearly that systemic reactivity is also abolished by desensitization.

Indeed, throughout the discussion of hypersensitivity and immunity the author's bias leads him into unfortunate inconsistencies, inaccuracies and logical faults. Thus he flatly rejects the work of the many investigators who have shown that tuberculous animals can be desensitized without loss of their immunity, insisting dogmatically that "no tuberculous animal has, as yet, been 'desensitized' or deprived of its allergy in any valid sense of the term" (page 118). When, however, he comes later to discuss one paper in which, in conformity with his own view of the significance of allergy, the experimental abolition of hypersensitivity was regarded as deleterious (page 146), he readily accepts those animals as having been desensitized and the work as being "fundamentally important," though the method, the criteria and the degree of desensitization in that experiment were precisely those used by the many other equally competent investigators whose opposite results he rejects. Furthermore, he does not mention that this same author, whose conclusions regarding a deleterious effect of desensitization in experimental animals he accepts, subsequently reported studies from which it was concluded that the desensitization of tuberculous patients was not deleterious but beneficial.

It should be clearly understood, however, that the aforementioned inadequacies in the treatment of immunologic principles and experimental data do not apply to the book as a whole. The more that Dr. Pinner knows by first hand experience of any aspect of tuberculosis about which he writes, the less dogmatic he becomes, and the more sound and balanced is his treatment of the subject; by far the greater part of the book falls within his own province of experience. It is only to be regretted that a book which in its clinical and pathologic portions is without doubt one of the finest and most valuable ever written on pulmonary tuberculosis should have been marred by capricious treatment of any of its fundamental sections.

The book is admirably illustrated and beautifully published.

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## DIAGNOSIS OF ERYTHROBLASTOSIS (HEMOLYTIC ANEMIA) IN THE MACERATED FETUS

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ON postmortem examination of a macerated fetus it is often difficult to determine whether erythroblastosis is present, and definite criteria for such a diagnosis have never been established. At the present time there is a widespread tendency to regard as having this disease all macerated fetuses from mothers whose red blood cells are shown not to contain Rh, the fact being overlooked that intrauterine death may occur in fetuses of such women from the same causes as function in women whose red cells contain Rh.

Among all infants who die before birth or in the first few days of life those who die before the onset of labor make up the majority of the group in whom a diagnosis as to the cause of death cannot be made from the necropsy or the clinical history. In a previously reported series of 2,000 infants who died in utero or during the neonatal period and on whom autopsies were performed,<sup>1</sup> those who died before the onset of labor in whom no cause of death could be ascertained made up 19 per cent. In 57 per cent of the antenatal deaths a cause could not be found. Rh determinations were not done on the mothers of these infants, but subsequent studies<sup>2</sup> have shown that among women who give birth to macerated fetuses in which death is due to causes other than erythroblastosis, the incidence of those whose erythrocytes do not contain an Rh factor is approximately the same as that among women in the general population.

The prognosis for future successful pregnancies is poor if a woman once bears an infant who dies of erythroblastosis. It is therefore extremely important to arrive at an accurate diagnosis. No woman should be urged to forego further pregnancies unless some definite evidence of the disease exists in the fetus in question.

Macerated fetuses in relation to this problem may be divided into three groups:

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1. Potter, E. L.: J. A. M. A. **115**:996, 1940.
2. Unpublished data.

1. Late premature or term fetuses in which maceration is slight. In these the same criteria which apply to nonmacerated fetuses and infants may be used.

II. Abortions; preivable or very premature fetuses. These almost never show a pathologic condition which can be diagnosed as erythroblastosis. If a woman has previously given birth to an infant with erythroblastosis, it is probably justifiable to believe that the same cause operated in causing the subsequent early intrauterine death. There is no positive proof, however, that this assumption is true. Abortion or early fetal death in women pregnant for the first time or in women who have never succeeded in carrying a child to term (the so-called habitual aborter) is almost never due to erythroblastosis.

III. Late premature or term fetuses with marked maceration. It is this group of infants with which this paper is particularly concerned, and it is believed that if careful postmortem studies are made, the presence or the absence of erythroblastosis can usually be established. Of especial importance is the presence of: edema, especially of the face; mild macroglossia; hypertrophy of the spleen and the liver; normal zone of growth at the ends of the long bones; enlargement of the placenta; erythroblastemia in placental vessels; basophilic erythroblasts in pulmonary capillaries; absence of Rh in the mother's erythrocytes and presence of Rh in the father's erythrocytes.

Even if the degenerative changes are advanced, erythroblastosis usually can be easily diagnosed in the typical case. All of the findings just listed will be present. Anasarca is frequently extreme and is often so severe that the abdominal wall is 1 to 2 cm. thick as a result of separation of the muscle layers by the edema of the intervening connective tissue. The tongue is edematous, and the tip protrudes through the separated lips (fig. 1 A). Pleural or peritoneal effusions may be present and, if large in amount, may compress the viscera against the posterior wall of the body. The organs of a macerated fetus weigh less than those of a nonmacerated fetus of similar size, but in spite of this the weight of the spleen is usually considerably increased (fig. 1 B). The liver is also frequently enlarged but not as markedly or as constantly as the spleen. The thymus is usually hypoplastic. The heart may be of normal size or slightly enlarged. At the ends of the long bones the zone of growth is normal.

The placenta is often greatly enlarged. At term the average placental weight without cord or membranes is about one-seventh that of the fetus. In erythroblastosis, it is frequently one-fourth as heavy as the fetus, and at times it may exceed half of the fetal weight. The enlargement is due to actual increase in the size of the individual villi brought about partly by edema and partly by tissue increase. Fluid



Fig. 1.—A, erythroblastosis in a macerated fetus and placenta. B, enlargement of the spleen and the liver of a fetus with erythroblastosis.

rapidly exudes from the placenta after delivery, and the weight must be determined at once if it is to be accurate. Increase in villous size produces a thick bulky placenta; fetal anemia plus villous edema gives less than the normal number of red blood cells per unit volume of placenta, so that the color is usually light pink in contrast to the dark red of the normal placenta. The cotyledons are unusually sharply demarcated.

The mother's erythrocytes are usually free of Rh. If they are not, the diagnosis must be regarded with suspicion no matter how typical the findings seem to be. Infrequently some other antigen, such as Hr, may be responsible for the manifestations of erythroblastosis, and a few cases have been described which were presumed to be due to A or B immunization. It is also possible for one of the subgroups of Rh to be responsible for sensitization, and a woman whose erythrocytes possess one Rh fraction may develop immunity to some other fraction which they lack. Except in rare instances, however, if the mother's red cells contain Rh, the course of subsequent pregnancies will show that the diagnosis of erythroblastosis was erroneous.

Syphilis is the principal condition with which erythroblastosis may be confused in a macerated fetus. Syphilis may duplicate any or all of the abnormalities which can be made out on gross anatomic examination. If sufficiently advanced to cause the aforementioned changes, syphilis, in addition, usually produces a thickened, irregular epiphyseal line at the ends of the long bones. The author has observed such a change in association with erythroblastosis of a non-macerated fetus in 2 cases in which histologic study, stains for *Treponema pallidum* and thorough investigation of the mother, father and other children failed to show evidence of syphilis. Such a change in the long bones is therefore not conclusive evidence of syphilis but its occurrence in any other condition is extremely rare. The presence of syphilis can usually be corroborated by the presence of the disease in the mother, the demonstration of *T. pallidum* in the tissues and the presence of histologic changes which differentiate the condition from erythroblastosis. It is also possible to have the two conditions coincident in the same fetus.

Another condition of extreme hydrosis occurs in the fetus that must be differentiated from erythroblastosis. It is associated with extreme anasarca, with pleural and peritoneal effusions and at times with placental hypertrophy, but the spleen is usually hypoplastic, the mother's red cells are usually free of Rh, and subsequent pregnancies end in the delivery of normal infants. Malformations are occasionally present. Eighteen such infants have been previously reported,<sup>3</sup> and

2 more have been subsequently observed. Nothing is known of the etiologic factors of this condition.

In contrast to the fetuses with typical erythroblastosis here described are those which have the disease in spite of showing little gross evidence that it is present. Not infrequently such fetuses appear normal on the cursory examination macerated fetuses are apt to receive. There may be little edema, and what is present may be mistaken for a change due primarily to maceration. Careful examination, however, usually reveals definite edema of the face, especially of the eyelids, even if it is otherwise absent. Protrusion of the tongue between slightly separated lips is common.

The internal viscera may reveal nothing abnormal, although in most instances some enlargement of the spleen will be observed. If the fetus has been dead for several weeks prior to delivery, even though the measurements may be abnormally great, the weight may be so reduced that it is within normal limits.

In such fetuses, histologic examination of the liver, the spleen, the kidneys and other organs, which is ordinarily so important in arriving at a diagnosis, is impossible. The rapid degeneration of the abdominal organs that occurs after death causes complete loss of cellular detail within a few days. Henderson<sup>4</sup> reported that he could demonstrate an increase in intralobular connective tissue in the liver in the majority of macerated fetuses with erythroblastosis. In my experience this increase is occasionally present (as it is also in nonmacerated fetuses and infants with erythroblastosis) but is not sufficiently constant or distinctive to be of much value. It is also frequently found in syphilis.

One organ, however, which is of great value even when extreme maceration is present is the lung. This tissue retains its affinity for dyes long after all other viscera have lost theirs, and some histologic details can be made out when the fetus is delivered even weeks after death has occurred. Of especial importance is the fact that erythroblasts and normoblasts are among the last cells to lose their affinity for stains (fig. 2A).

An interest in the contents of the pulmonary alveoli of macerated fetuses originally led to routine sectioning in this laboratory of all lungs regardless of the degree of maceration. It soon became evident that erythroblastosis which was previously unsuspected could be occasionally diagnosed from sections of lung. Examination of the lungs of over a thousand macerated infants has failed to reveal in any other condition the findings about to be described.

In the lungs of both macerated and nonmacerated fetuses an interesting picture presents itself. Local erythropoiesis almost never occurs.

4. Henderson, J. L.: Arch. Dis. Childhood 17:49, 1942.

Even in the interstitial tissue around the blood vessels, where in comparable places in other organs erythropoiesis may be marked, there is little or no evidence of blood cell formation. Within the capillaries of the alveolar walls, however, there are large numbers of the most immature forms of red blood cells, cells which in blood smears are

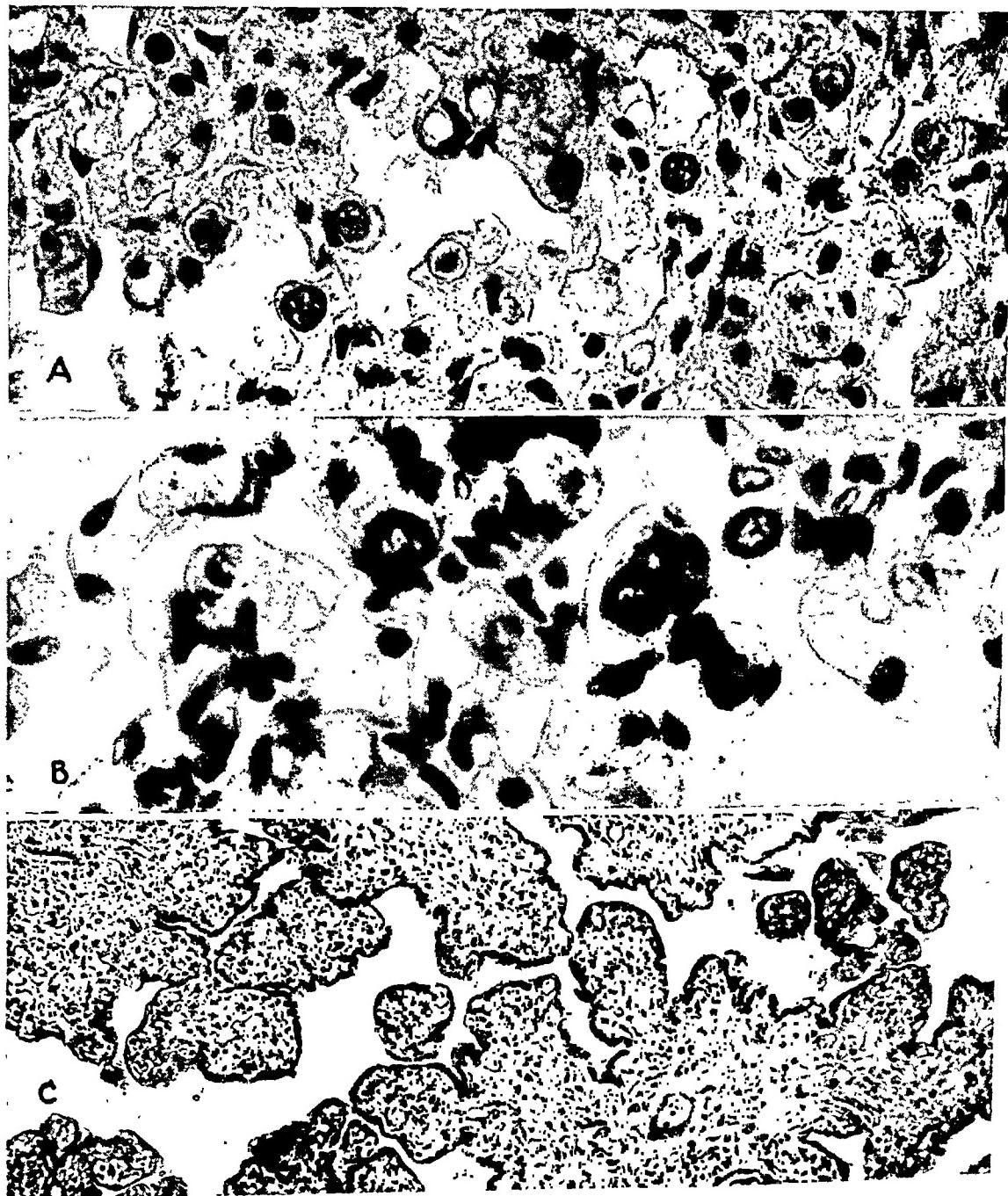


Fig. 2.—*A*, macerated lung with erythroblasts in pulmonary capillaries. *B*, non-macerated lung with erythroblasts in pulmonary capillaries. *C*, placenta showing typical shape of villi in erythroblastosis fetalis.

variously called basophilic erythroblasts, megaloblasts and hemocytoblasts by different investigators (fig. 2*B*). They are large in relation to the normal caliber of the pulmonary capillaries, and the appearance suggests that the cells are strained out of the general circulation and lodged in these vessels. It seems conceivable that these cells might interfere sufficiently with pulmonary circulation to be a factor in the death of some infants with this disease. An abnormal number of immature red blood cells in all stages of development are also found in the clots in the larger blood vessels, and although the cells often cannot be specifically identified, the fact that a disproportionately large number of nuclei are present is easily recognizable.

The erythroblasts within the pulmonary capillaries are early cells with large nuclei and little cytoplasm. They can ordinarily be easily distinguished from septal and connective tissue cells of the alveolar walls by their greater nuclear size and superior ability to retain dyes. These cells are characteristically found in this location only in the fetus and the newborn infant with erythroblastosis. Consequently the lung of the macerated fetus is ordinarily the one organ which will give histologic evidence of the disease. If only one tissue were available in the case of a macerated or nonmacerated fetus or liveborn infant, I believe a correct diagnosis could be made from the lung in a higher percentage of cases than from any other organ.

If the placenta is available, it is of great aid in establishing a diagnosis. When an infant is born alive with erythroblastosis, the placenta may show no gross or histologic deviation from normal, but if the disease is sufficiently severe to cause intrauterine death, the placenta is almost always abnormal. The total size and the weight are increased, and the cotyledons are large, thick and unusually well demarcated. The placenta undergoes degenerative changes much more slowly than the fetus, and frequently the villi are well preserved and can be subjected to histologic study long after this becomes impossible in most fetal organs. The villi are frequently enlarged (fig. 2*C*). The stroma is edematous, and after fixation the loss of fluid from the interior may cause a loosening of the outer covering. Langhans' cells are often more numerous than they normally are and at times form an almost continuous layer of large hydropic cells beneath the syncytial covering. The cellularity of the stroma is often increased. The blood vessels are usually found near the peripheral margins of the villi, and although they may contain few blood cells, some immature red cells are almost always present. The vessels in the major villi frequently contain clots showing large numbers of nucleated cells.

## SUMMARY

It is often difficult to arrive at a definite conclusion concerning the cause of death in a fetus that has died before the onset of labor. Careful gross examination combined with histologic study of the lungs will usually make it possible to diagnose erythroblastosis if it is present. The occurrence of erythroblasts in the pulmonary capillaries is the most important single diagnostic sign to be found in the macerated fetus. The placenta adds valuable corroboratory information and, if available, should always be examined.

The failure to demonstrate Rh in the erythrocytes of the mother is by itself insufficient evidence on which to base a diagnosis of erythroblastosis. Even in the presence of maceration a definite diagnosis can usually be made, but if any doubt exists, a positive diagnosis should be withheld. Unless there is conclusive evidence of erythroblastosis, the mother should not be advised against future child bearing.

## HYPERTENSION AND NECROTIZING ARTERITIS IN THE RAT FOLLOWING RENAL INFARCTION

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AN EARLIER paper<sup>1</sup> presented a description of the circulating blood returning to infarcted renal tissue through reestablished vascular structures in the rat, and in conclusion it was mentioned that studies were in progress concerning the morphologic and functional alterations which result when the blood flows through such injured and dead tissue. These studies are described in the present report. Since they were undertaken to investigate the correspondence, if any, of functional or structural abnormalities occurring in the body of the rat with changes taking place in the renal infarct, the findings of that earlier work are briefly summarized here.

It was shown that soon after ligation and severance of a large branch of the renal artery it is possible for blood to enter the most distal glomeruli and arterioles of the severed artery from the neighboring tissue with an uninterrupted blood supply by way of the communicating intertubular capillary network of the two areas. After three days the main arterial stems of the infarcted area, as well as many of its glomeruli and intertubular capillaries, are open to the general circulation. At one week many more capillaries are patent, while at two weeks an extension of blood flow is observed in the previously only partly patent interlobular arteries, which in the interval have united to some extent with the intertubular network. At one month blood can flow throughout the entire necrotic mass in a continuously united sparse network of small vessels which join the main arteries and veins. Gradual absorption and shrinkage of the necrotic tissue occur. The shrinkage affects the vessels as well as the interstitial tissue, so that at the end of a year the area of infarction is a thin scar containing some fibrous tissue but consisting mainly of a vascular tissue which is recognizable as an evolution of the original vascular structures. The vessels joining the vascular system of the scar with that of the neighboring tissue are enlargements

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1. Loomis, D., and Jett-Jackson, C.: Arch. Path. 33:735, 1942.

of the intertubular network which connects the glomeruli and arterioles of the two areas.

Since there are these communications, however small at the beginning, between the vessels of the neighboring uninvolved tissue and those of the infarct, it is possible for blood to flow into the affected area and degeneration products to drain from it into the venous system from the earliest moment of damage. Contact between the necrotic tissue and the circulating blood extends at an accelerating rate as blood flows through the increasing number of newly established or cleared pathways. This contact reaches a maximum and then diminishes with the shrinking of the necrotic mass by absorption. Throughout these continuously changing phases there must be quantitative and qualitative variations in the physical and the chemical structure of the dead tissue. Indeed, two such changes can be observed histologically, i. e., the precipitation of calcium, especially in the uppermost portions of the cortex, and the appearance of fat, particularly in the necrotic material of the glomeruli and proximal convoluted tubules.

On the basis of these facts the questions which I wish to explore in this study are:

1. Does renal infarction have effects on the circulatory system of the animal as evidenced by a functional disturbance, such as hypertension, or by structural changes, such as arterial lesions?
2. If so, can it be assumed that these effects are due to an agent absorbed from the infarcted area? What is the relation between the rate of development of the circulatory contact with the necrotic mass and the absorption of this and the rate of appearance and the degree and the duration of functional and structural changes?
3. Since infarction of necessity reduces the amount of renal tissue, does the reduction of this tissue account for some of the effects noted?
4. If so, what is the relative importance of the two factors, absorption of necrotic tissue and reduction of renal parenchyma?

#### EXPERIMENTAL MATERIAL

The rats used in the experiments weighed from 150 to 225 Gm. They were fed exclusively on Purina dog chow and given tap water to drink ad libitum. They fall into the following categories according to the experimental procedure:

*Series 1* (rats with 100 per cent renal substance).—These were normal controls, with no operation; i. e., their kidneys were intact and free of infarction and are represented in charts and tables by two blank circles.

*Series 2* (rats with 75 per cent renal substance).—These animals had undergone operative infarction of one half of one kidney, indicated in charts and tables by blackening of half of one of the circles.

*Series 3* (rats with 50 per cent renal substance).—The animals were grouped according to the operations which they had undergone: (a) unilateral nephrectomy,

indicated by a single blank circle; (b) total infarction of one kidney, indicated by blackening of one of the circles; (c) infarction of one half of each kidney, indicated by blackening of half of each circle.

*Series 4* (rats with 25 per cent renal substance).—These rats also were grouped according to the operations which had been performed on them: (a) subtotal nephrectomy-extirpation of one kidney and of half of the other, indicated in charts and tables by one-half blank circle; (b) subtotal nephrectomy and infarction. The operation included extirpation of one kidney and infarction of one half of the other, indicated by one circle, half blackened; (c) infarction of 75 per cent of renal tissue, infarction of one kidney and of one half of the other, indicated in charts and tables by one blackened circle and one half-blackened circle.

The circles mentioned as used in charts and tables represent kidneys; the blackened portion shows the amount of infarction.

#### OPERATIVE PROCEDURE

The technic used was the same as that described in a previous publication.<sup>1</sup> In every case of infarction the renal artery or one of its main branches was isolated, ligated in two places and cut between the ligatures. In group 4a (subtotal nephrectomy leaving one-half kidney) the blood flow was arrested temporarily by placing a bulldog clamp on artery and vein while the poles were excised; the blood flow was restored immediately afterward by removing the clamp. The cut surface of the kidney was not cauterized.

#### MEASUREMENTS OF BLOOD PRESSURE

The blood pressure was measured in all animals by the indirect method of Byrom and Wilson<sup>2</sup> with some slight modifications. Glass was used instead of cellophane for the pressure chamber and Penrose tubing for the cuff. Also the procedure of measuring the blood pressure was varied somewhat. The rat's approximate blood pressure was roughly established and then carefully measured by raising the mercury level only 10 to 20 mm. above the expected point and letting it fall slowly until the sharp rise occurred in the plethysmograph. This was done to prevent collapsing the vessels by excessive pressure for periods long enough to cause retardation of the returning blood in the capillary bed.<sup>3</sup> Ether anesthesia was used.

It was discovered in trial tests that since the blood pressure falls during anesthesia and rises as the rat emerges, the point which most truly represents the animal's blood pressure is uncertain. One reading is inadmissible, and an average of varying readings has no particular validity either, so that in the end the standard procedure described in the following paragraph was adopted. This procedure, though arbitrary, nevertheless permitted repetition and control and could be applied to every animal.

The animal was anesthetized sufficiently so that it could be adjusted to the apparatus and was laid on a cork platform kept warm by an electric lamp set at a standard distance above the animal. After the plethysmograph was adjusted, repeated measurements were taken until the rat's movements as it came out of anesthesia made it impossible to take more. The animal was then anesthetized again for sixty seconds, and readings were repeated until it began to move.

2. Byrom, F. B., and Wilson, C. J.: *J. Physiol.* **93**:301, 1938.

3. Griffith, J. Q., and Farris, E. J.: *The Rat*, Philadelphia, J. B. Lippincott Company, 1942.

It was anesthetized a third time for sixty seconds, and measurements were repeated as before. An average of the five highest readings made while the animal was quiet in the three periods of anesthesia was the final figure taken for the blood pressure. Usually these five readings were within 1 to 6 mm. of each other, and in general the readings in each period repeated closely those of the other two. The readings within any one period, however, might vary from 10 to 20 mm. Kempf and Page<sup>4</sup> stated that with their method, a modification of that of Williams, Harrison and Grollman,<sup>5</sup> departures as great as 100 mm. from the average were noted. I found no such extreme variation, though sick animals may show a wider range than normal animals, the blood pressure either continuously dropping on successive anesthetizations or continuously rising.

Blood pressure measurements were made on all rats on at least one but usually on two occasions before operation, twenty-four hours later, and at weekly intervals thereafter. The blood pressure of a few animals was also measured at six, twelve and eighteen hours after operation and then daily during the first week.

#### DURATION OF EXPERIMENT

Animals were kept until they died, or if they lived for a year after operation, they were killed.

#### POSTMORTEM EXAMINATION

An autopsy was made on every rat, and sections of heart, lungs liver, spleen kidneys, intestines, pancreas and mesentery were fixed in both Zenker's fluid and a 10 per cent solution of formaldehyde. Hematoxylin and eosin and in some cases Masson and Weigert stains were made on the sections for microscopic study.

#### GENERAL OBSERVATIONS

Differences were observed in the several groups as regards blood pressure, pathologic changes and length of life.

*Blood Pressure.*—In normal rats the blood pressure when measured by these methods was found to vary from 74 to 156 mm. of mercury, with a mean of 110. Byrom and Wilson<sup>2</sup> estimated the normal pressure at 78 to 132 mm. of mercury, with a mean of 106. Figure 1 shows the findings of Williams, Wegria and Harrison<sup>6</sup> in regard to 1,207 normal rats when using an indirect method with unanesthetized animals, compared with all the preoperative readings of the experimental rats used in this study combined with all the weekly readings of the 11 controls, most of which lived a year or nearly a year after the experiments were started.

It was hoped by using ether anesthesia to eliminate the variability in blood pressure due to animal excitement. Day to day measurements showed only slight variation in normal animals; in weekly measurements the swings were more marked but had a tendency to follow a curve of

4. Kempf, G. F., and Page, I. H.: J. Lab. & Clin. Med. **27**:1192, 1942.

5. Williams, J. R.; Harrison, T. R., and Grollman, A.: J. Clin. Investigation **18**:373, 1939.

6. Williams, J. R.; Wegria, R., and Harrison, T. R.: Arch. Int. Med. **62**: 805, 1938.

gradual rather than precipitous increase and decrease. The blood pressure in some of the controls remained at a remarkably constant level for many weeks. Indeed, that of 1 animal varied less than 10 mm. in twenty weeks and not more than 5 mm. in eight weeks. No normal control animal showed throughout the entire year an elevation of more than 25 mm. above the initial blood pressure measurement, and the highest measurement of blood pressure obtained from controls was 158 mm., and this occurred only once. Therefore any rat with an elevation of blood pressure above 160 mm. of mercury or which showed an increase of more than 25 mm. above the preoperative level is considered hypertensive in these experiments.

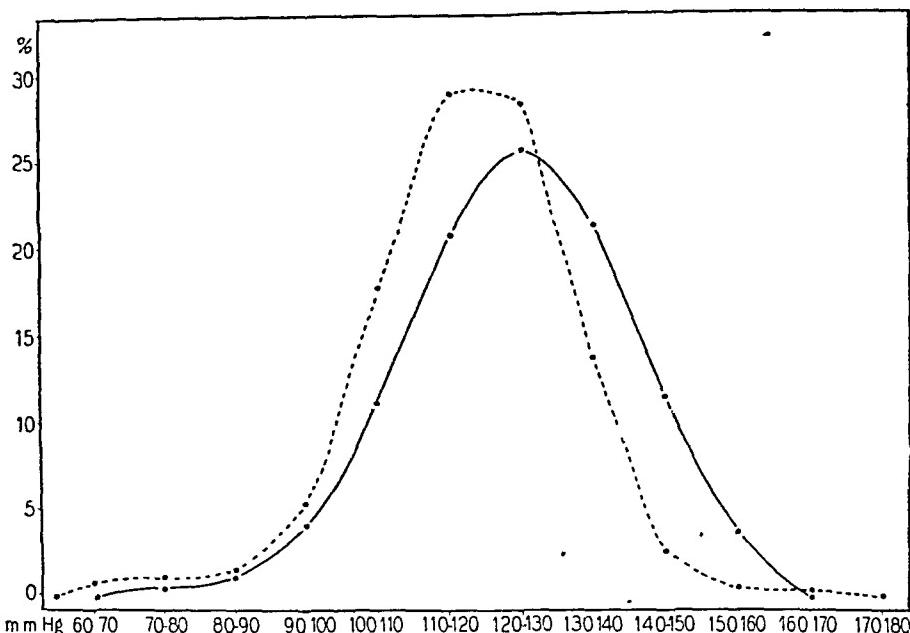


Fig. 1.—Curves showing the range of blood pressure in normal rats as determined by Williams, Wegria and Harrison<sup>6</sup> from the measurements of 1,207 animals (----) and as determined in the present study from 647 measurements of 100 animals (—).

Hypertension developed in a high percentage of the rats with renal substance reduced, whether by infarction or by extirpation (fig. 14). Only a general statement of certain characteristics of the hypertension needs to be made at this time, for the details of the effect of each of the various operations on blood pressure will be analyzed later. Hypertension did not develop in most animals until after some weeks (see table 1); therefore no animals that died in less than three weeks are included in the consideration of hypertension. The highest blood pressure measured was 262 mm. of mercury, and pressures above 220 mm. were not uncommon. The hypertension which persisted longest was observed to the end of the experiment at one year. In some hypertensive rats

the pressure declined to lower levels or became normal before the end of the experiment. Sharp drops and elevations occurred fairly frequently, and often a precipitous fall to a very low level immediately preceded death. Differences were also observed in the rate of development of hypertension and in the general form of the blood pressure curves with the duration of the experiment, and these differences appear to have significance.

It is evident that critical evaluation of such varying data is possible only when the evidence is available in its entirety. For this reason all the characteristics of the blood pressure readings may be seen in the complete data presented in figures 8 to 15, inclusive. Here the individual blood pressure curve of each rat is compared with the curve of the

TABLE 1.—*Development of Hypertension in Rats with Renal Substance Reduced*

Weeks After Operation		Partial Infarction				Total Infarction
		Group 2 ○○	Group 3c ○○	Group 4b ○	Group 4c ○○	Group 5b ○●
		Number of rats in group	10	10	11	10
1	Number hypertensive	6	9	10	7	2
2	Number of rats in group	10	10	11	10	12
	Number hypertensive	6	9	10	8	2
3	Number of rats in group	10	10	11	10	12
	Number hypertensive	10	10	10	9	2
4	Number of rats in group	10	10	11	8	11
	Number hypertensive	10	10	10	7	4
5	Number of rats in group	10	10	10	6	11
	Number hypertensive	10	10	10	6	5
6	Number of rats in group	10	9	8	5	11
	Number hypertensive	10	9	8	5	6

weekly average of the experimental group and the curve of the weekly average of the normal control group. Typical single curves are also presented.

*Vascular Lesions.*—Vascular lesions similar to those observed in rats by Wilson and Byrom<sup>7</sup> after compressing a renal artery, by Friedman, Jarmon and Klemperer<sup>8</sup> after applying cellophane to one kidney and by others<sup>9</sup> after performing similar procedures on rats or other animals occurred frequently in several groups.

7. Wilson, C. J., and Byrom, F. B.: Quart. J. Med. **10**:65, 1941.

8. Friedman, B.; Jarmon, J., and Klemperer, P.: Am. J. M. Sc. **202**: 20, 1941.

9. (a) Cromartie, W. J.: Am. J. M. Sc. **206**:66, 1943. (b) Child, C. G.: J. Exper. Med. **67**:521, 1938. (c) Goldblatt, H., and Kahn, J. R., in Publication 13, American Association for the Advancement of Science, 1940, p. 266. (d) Wilson, C., and Pickering, G. W.: Clin. Sc. **3**:343, 1938. (e) Smith, C. C.; Zeek, P. M., and McGuire, J.: Am. J. Path. **20**:721, 1944. (f) Schroeder, H. H. and Neuman, C.: J. Exper. Med. **75**:527, 1942.

The lesions were most frequent and most extreme in degree in the mesenteric vessels but occurred also, in severely affected animals, in all of the other abdominal and pelvic arteries except the aorta. The smaller arteries and arterioles of the heart and the kidney were next most frequently affected, while those of the liver, the spleen and the lungs were involved only rarely.

The skeletal muscles and the central nervous system were not examined, so that the incidence of the vascular lesions in those areas is not known. Cyanosis of the foreleg developed in 1 animal, and dissection on its death revealed that the brachial artery was extensively involved.

In extreme examples the affected arteries were strikingly altered (fig. 2 A). The walls were irregularly thickened and nodular in appearance, with aneurysmal dilatations and tortuosities. On section they were sometimes found to contain thrombi. In animals which died late in the course of the experiments or which were killed at one year the walls were fibrous and occasionally calcified. The process sometimes was confined to one or two fibrous or calcified nodules found exclusively in the vessels of the tissue attaching the pancreas and the duodenum (and could have been easily missed unless searched for).

Microscopically, the lesion in its acute stage was seen to be a necrotizing and inflammatory process involving all three coats of the vessel. It was characterized by necrosis of the intima (fig. 2 B and C), deposits of subintimal hyaline or fibrinoid masses which often extended into the media, necrosis of the muscle cells of the media (fig. 2 B, C and D) and shredding and disappearance of the elastic tissue (fig. 3 B). All coats were progressively infiltrated with quantities of cells. These were neutrophilic leukocytes, many eosinophils, and mononuclear cells with ovoid nuclei containing deeply basophilic chromatin masses (figs. 2 D and 3 A). In acute lesions thrombi were often fused with the fibrinoid material of the intima. The lesion progressed to a granulomatous stage, fibroblasts and capillaries penetrating to the intima, sometimes in a radial arrangement, while in the outer portions the young connective tissue and capillaries were more or less concentrically arranged (fig. 3 A). In the granulation tissue there were lymphocytes and plasma cells. Hemorrhages were present in the involved arterial wall, and deposits of hemosiderin were common.

In some arteries the inflammatory condition appeared to subside with little residue beyond fibrosis of the adventitia. In others, the muscle cells did not regenerate, and the walls were composed entirely of connective and elastic tissue. The elastic tissue either fragmented and disappeared (fig. 3 B) or was increased and dispersed throughout the whole wall as in figure 4 A. The necrotic areas in the media were

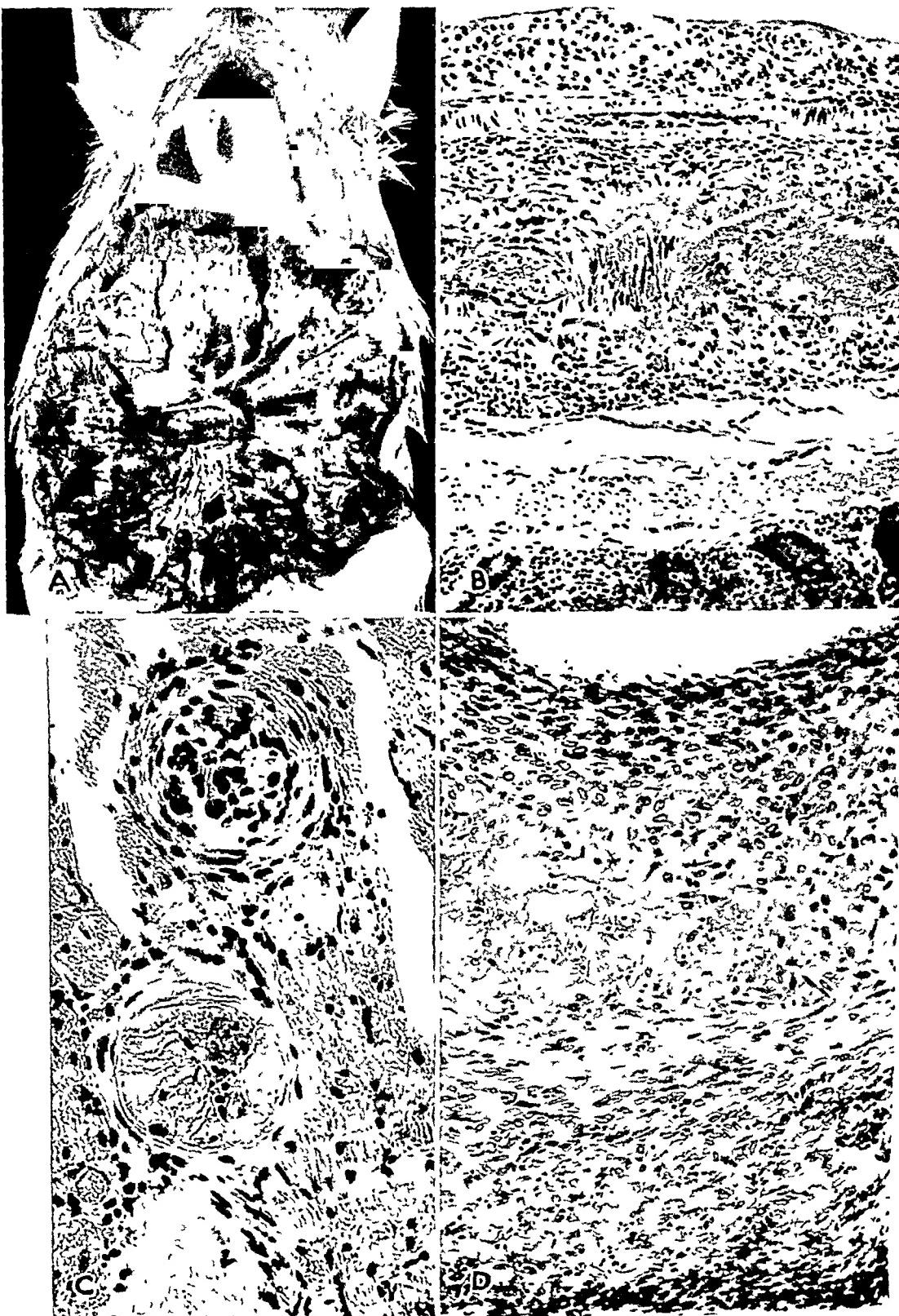


Figure 2  
*(See legend on opposite page)*

not infrequently the seat of calcium deposits (fig. 4 *B*; compare with fig. 2 *D*). On healing, the granulation tissue in and around the adventitia became in the end dense collagenous tissue (fig. 4 *D*), the thrombi were organized and the lumens of the arteries were completely occluded.

Other modifications in the structure of the arteries and the arterioles took place, such as proliferation of the endothelium which almost filled the lumen (fig. 2 *C*), focal proliferation of subendothelial connective tissue with elastic tissue ramifying between the connective tissue cells (fig. 5 *A*) and hypertrophy of the media (fig. 5 *B*).

These changes in some cases appeared to be arrested and healed; in others healing and new lesions were encountered even as late as one year after the experimental infarction, the acute lesions being superimposed on an older process.

*Parenchymal Lesions.*—(a) Heart: In severe involvements the lesions of the cardiac muscle could be detected with the naked eye, appearing as white patches with linear streaks. The lesions were most commonly found in the right auricle but were also observed in the left ventricle and frequently at or near the apex.

Microscopically, these streaks were seen to be areas of necrosis of muscle fibers, in which there was sometimes hemorrhage and into which many cells of mononuclear type had infiltrated (fig. 5 *C*). Plasma cells and lymphocytes were present, but the chief cell was one with a large round or ovoid nucleus and little cytoplasm. Polymorphonuclear cells were usually absent or, if present, were few. Necrotizing arteritis and arteriolitis were observed in the coronary vessels, and these vascular lesions were often, but not always, seen in close relation to the lesion of the muscle.

Sometimes infiltration with mononuclear cells was observed in the absence of necrosis of the cardiac muscle cells, and among the infiltrating cells were some of the type known as Anitschkow myocytes. The latter

#### EXPLANATION OF FIGURE 2

*A*, gross aspect of the lesions of the mesenteric arteries, showing the nodular thickening, tortuosity and dilatation. Rat P-15 of group 4 *c* (one kidney and half of the other showing complete infarction) at seventeen weeks.

*B*, mesenteric artery showing necrosis of media and intima. The subintimal fibrinoid mass is fused with that in the lumen. There is a periarterial infiltration of inflammatory cells. Rat P-59 of group 3 *c* (one half of each kidney showing complete infarction) at one week.  $\times 150$ .

*C*, necrosis of arterioles in the heart muscle. The fibrinoid mass completely fills the lumen of each of the lower vessels. The vessel at the top shows proliferation of the endothelium. Rat P-49 of group 4 *c* (one kidney and half of the other showing complete infarction) at four weeks.  $\times 300$ .

*D*, mesenteric artery showing necrosis of media, proliferation of endothelium and infiltration of all coats with inflammatory cells. Rat P-88 of group 4 *c* (one kidney and half of the other showing complete infarction) at three weeks.  $\times 150$ .



Figure 3  
*(See legend on opposite page)*

were also seen in small collections in the endocardium and in the adventitia of the coronary vessels in arrangements resembling Aschoff bodies (fig. 5 D). Fibrous tissue proliferation ramified through the involved areas, and in later periods fibrous scars of varying sizes were observed in the myocardium.

(b) Kidney: The changes in the uninfarcted portion of the renal tissue aside from hypertrophy varied from an occasional scar and a few dilated tubules to extreme damage, as seen in figures 6 B and C. Such extreme changes were observed only in those three groups which had had the renal substance reduced to 25 per cent of the original total by infarction or excision or both, and in only a portion of the animals in these groups. Rats of these groups dying early showed beginning dilatation of tubules and cast formation as early as five weeks after operation (fig. 6 A), and these changes became progressively more marked in rats dying later. The extreme distention of the tubules with masses of hyaline material such as is shown in figure 6 B and C was not observed until eighteen weeks or later. In a few animals of each of the three aforementioned groups the renal lesion did not develop, the remnant of kidney apparently compensating sufficiently to prevent it.

The characteristic changes in the tubules of the nephrons are therefore the so-called degenerative lesions, consisting of cystic dilatation of the tubules, distention by casts, parenchymatous and fatty degeneration, compression atrophy of the epithelium in the distended tubules and desquamation of epithelial cells.

The altered glomeruli in the involved regions were large, pale staining and anemic and often completely filled Bowman's capsule. The capsular space, if not obliterated, was dilated and filled with coagulated material, and the capsular membrane was swollen and thickened. There was little or no blood in the capillaries of the tuft, and its tissues showed fewer nuclei than usual, while the epithelial cells of its membrane were large and swollen and contained foamy cytoplasm (fig. 6 D and E). Small aggregations of cells proliferating at one side of the capsule and somewhat resembling a glomerular crescent were observed in some cases.

#### EXPLANATION OF FIGURE 3

*A*, massive increase in the thickness of the wall of a mesenteric artery. Little of the original structure is left. Note that periarterial fibrosis, infiltration of granulation tissue with inflammatory cells and fibrinoid necrosis can all be found in this section. Rat P-10 of group 4 c (one kidney and half of the other showing complete infarction) at thirteen weeks.  $\times 133$ .

*B*, section of three branches of a mesenteric artery. The vessels at the right show almost complete disappearance of elastic tissue; the one at the left shows this tissue in fragmented and widely scattered strands. Rat P-32 of group 2 (one half of one kidney showing complete infarction) at fifty-two weeks.  $\times 53$ .

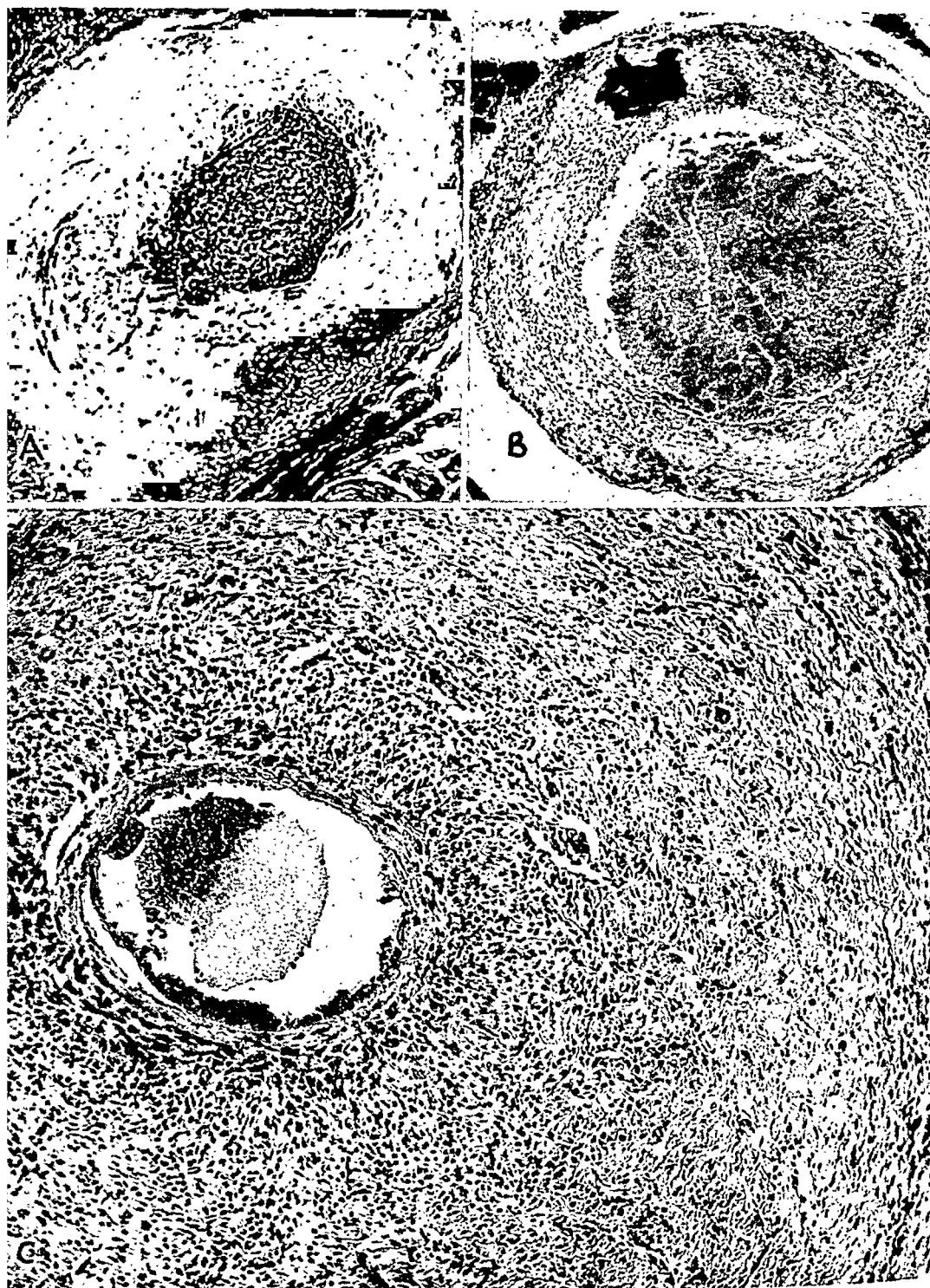


Figure 4  
*(See legend on opposite page)*

Necrotizing arteritis and arteriolitis also occurred in the kidney (fig. 6 F). They were noted almost entirely in the groups of animals with infarcted kidneys and were not seen in the group with unilateral nephrectomy, nor in the controls. They were seen as early as one week and as late as one year and in kidneys with and without the parenchymal changes described in foregoing paragraphs. Infiltration of the arterial coats with inflammatory cells was not present in the intrarenal arteries, and no fresh infarcts or wedge-shaped scars of infarcts that could have been the result of these vascular lesions were found.

Besides the acute necrotizing vascular lesion just described, marked hyaline thickening of the walls of the interlobular arteries and afferent arterioles occurred in some cases, the lumens of the vessels being almost obliterated. This was observed in the kidneys of those rats which had great reduction of renal substance, high blood pressure and marked parenchymatous changes in the renal remnant. Focal lymphocytic infiltrations and some increase in interstitial connective tissue occurred around the atrophic tubules and glomeruli in these kidneys.

The appearance of the infarcts that had been produced to initiate the experiment varied according to the time of death of the animal. Examination of kidneys of rats dying at fifteen and eighteen weeks showed that absorption of necrotic debris was not yet complete. By twenty-six weeks the tissue was about as condensed as at fifty-two weeks, with nothing remaining but calcium masses, a little connective tissue, blood vessels and a few remnants of collecting tubules. Atrophic but well preserved glomeruli persisted at the borders of the infarct.

*Length of Life After Operation.*—Death not infrequently occurred soon after operation in those groups which had undergone a double operation and which were deprived of most of their renal tissue. Of the total number of 100 rats operated on, 17 lived a week or less, and 5 lived between two and three weeks. These were not included in the consideration of length of life after operation, only such animals being

#### EXPLANATION OF FIGURE 4

*A*, dense and irregular spread of elastic tissue throughout the wall of a mesenteric artery. Rat P-32 of group 2 (one half of one kidney showing complete infarction) at fifty-two weeks.  $\times 53$ .

*B*, deposit of calcium in the media of a pancreatic artery. Compare the area of deposition with the area of necrosis shown in figure 2 D. Rat P-62 of group 2 (one half of one kidney showing complete infarction) at fifty-two weeks.  $\times 53$ .

*C*, mesenteric artery showing collagenization of periarterial tissue and the presence of many capillaries in the vessel wall. The original vascular tissue has been completely replaced by connective tissue. Rat P-70 of group 4 b (one kidney extirpated and half of the other showing complete infarction) at twenty-four weeks.  $\times 133$ .

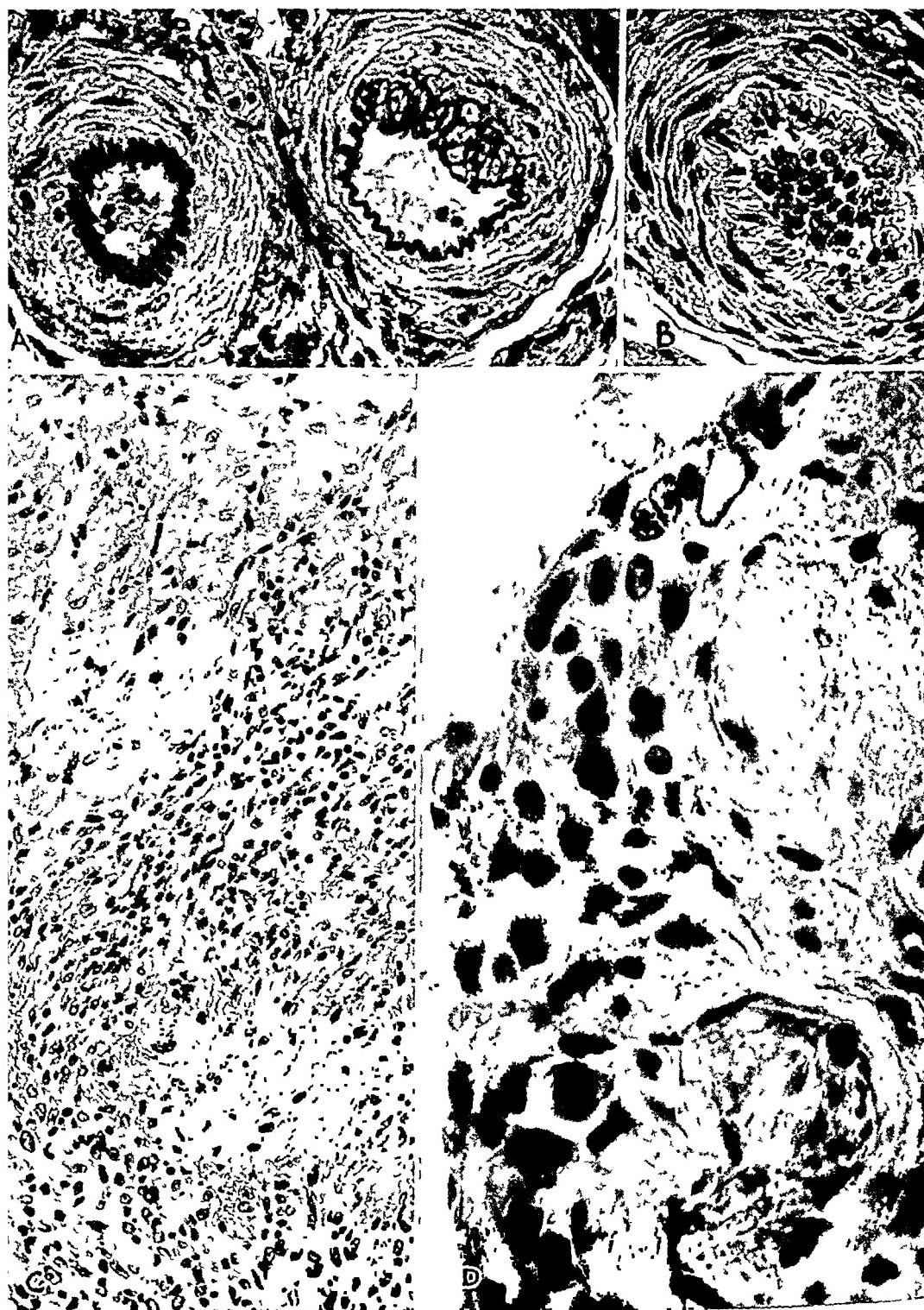


Figure 5

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used for this purpose as had lived three weeks or longer, a period corresponding to the limits set for the consideration of hypertension. It was felt that the effect of intercurrent disease on the length of life after operation as well as on blood pressure was too indefinite a quantity to assay, although it was quite obvious at autopsies that the deaths of some animals were due at least partly to such conditions as chronic pneumonia with bronchiectatic abscesses, acute pneumonia, cecitis and neoplasms. However, deaths which could be ascribed to those conditions occurred chiefly in the latter part of the experiments and do not have the significance of the earlier deaths. The controls, as well as the animals used in the experimental procedures, showed these diseases. None of the controls, however, exhibited the condition reported by Moore and Hitchcock<sup>10</sup> and called chronic nephrosis by Saxton and Kimball<sup>11</sup> or the vascular disease found in old animals by Wilens and Sproul.<sup>12</sup> The oldest animals were approximately 18 months old at the time the experiments were terminated.

The deaths at various intervals throughout the experimental period appeared roughly to group themselves into those occurring in an early and those in a late stage with six months, i. e., twenty-six weeks, as the dividing line. In the latter group are included the rats which survived to the end of the experimental period of one year and were then killed. The distribution of deaths is shown in figure 7.

There were seven accidental deaths due to overanesthetization. When it is considered that this small number occurred in more than 3,000 opportunities, the risk of the method does not seem great, and even these few deaths could have been avoided by closer attention. Since most

10. Moore, R. A., and Hitchcock, F. A.: Proc. Soc. Exper. Biol. & Med. **27**:206, 1930.

11. Saxton, J. A., and Kimball, G. C.: Arch. Path. **32**:951, 1930.

12. Wilens, S. L., and Sproul, E. E.: Am. J. Path. **14**:177 and 201, 1938.

#### EXPLANATION OF FIGURE 5

*A*, small vessels of the mesentery showing thickening of their walls. The one at the right show elastic tissue fibrils ramifying was in the focal proliferation of subendothelial tissue. Rat P-32 of group 2 (half of one kidney showing complete infarction) at fifty-two weeks.  $\times 250$ .

*B*, hyperplasia of muscle cells in the media of a mesenteric arteriole. Rat P-32 of group 2 (half of one kidney showing complete infarction) at fifty-two weeks.  $\times 375$ .

*C*, microscopic view of a lesion in the myocardium. Note an area of necrosis of muscle cells with infiltration of inflammatory cells. Rat P-59 of group 3c (half of each kidney showing complete infarction) at one week.  $\times 250$ .

*D*, cellular aggregation in endocardium resembling an Aschoff body. Rat P-49 of group 4c (one kidney and half of the other showing complete infarction) at four weeks.  $\times 666.5$ .

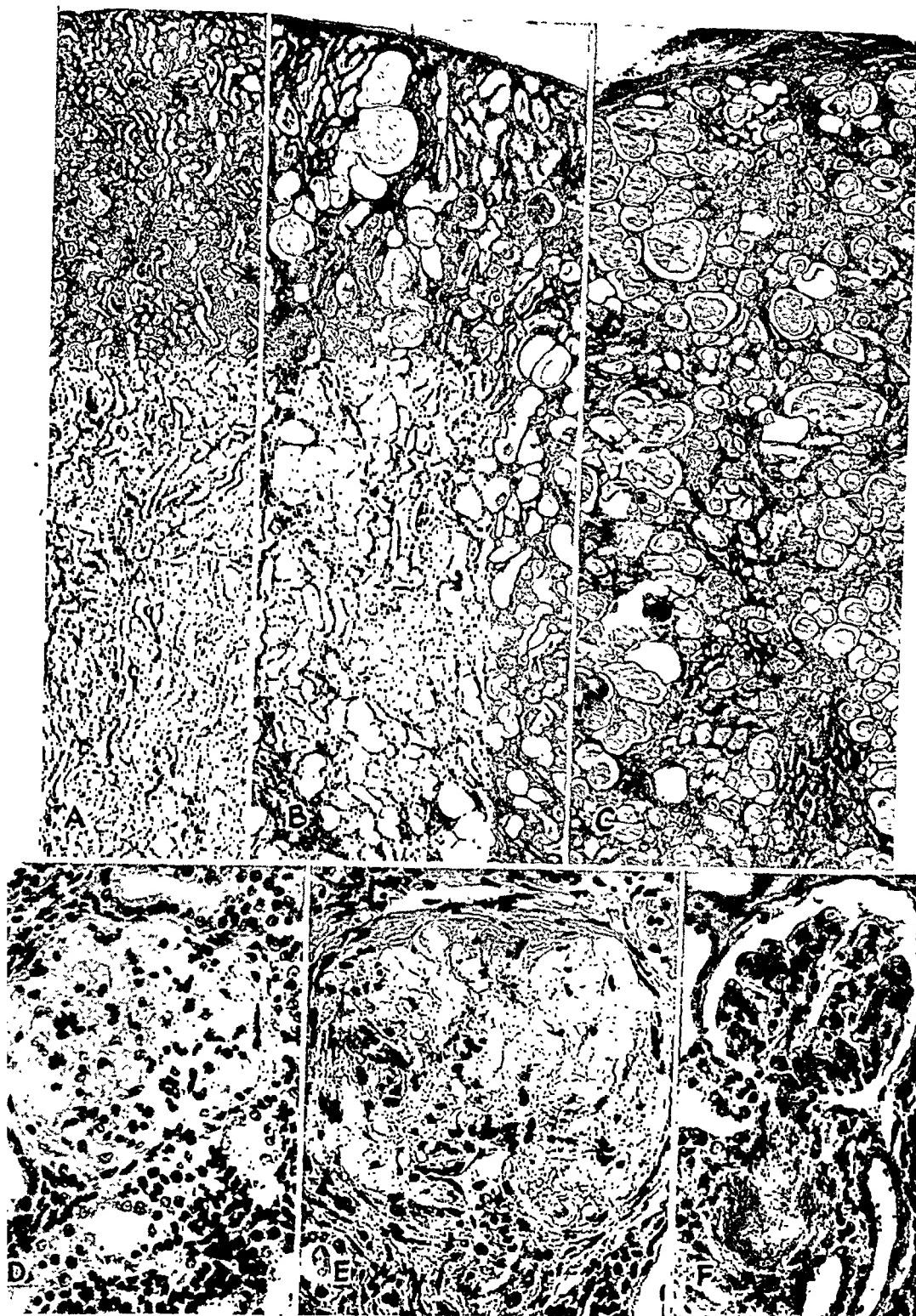


Figure 6

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of them occurred late in the experimental period, the deaths have no great weight in the percentages presented in figure 14.

#### RESULTS IN EACH EXPERIMENTAL GROUP

*Series 1* (controls: animals with 100 per cent of their renal substance).—In the controls, with no operation, the blood pressure was never elevated above 160 mm. of mercury, though in later months there was a tendency in some for it to be slightly elevated above the original level. A curve which is typical of this group is shown in figure 8A; all the individual and the mean curves for the group are shown in figure 9A. There were no arterial lesions. One animal, or 9 per cent of these rats, died before six months; 54 per cent lived one year.

*Series 2* (animals with 75 per cent of their renal substance, one kidney being one-half infarcted).—In 100 per cent of the animals the blood pressure was elevated above 160 mm. of mercury, and 30 per cent showed blood pressures above 200. After a sustained elevation of several months, although there was some variability in the curves, there was a general tendency toward a drop in blood pressure. This is shown in a typical animal in figure 8B. All the individual and the mean curves for the group are shown in figure 9B. Fifty per cent

#### EXPLANATION OF FIGURE 6

*A*, section of kidney of a rat deprived of 75 per cent of its renal tissue by infarction. Note the beginning dilatation of tubules and the granular precipitate in their lumens. Rat P-16 of group 4c (one kidney and half of the other showing complete infarction) at five weeks.  $\times 26$ .

*B*, section of kidney of a rat deprived of 75 per cent of its renal tissue partly by infarction and partly by extirpation. The greatly dilated tubules have flattened epithelium and contain masses of hyaline material. In some areas, the tubules are atrophic, and there is an increase in connective tissue. Rat P-29 of group 4b (one kidney extirpated and half of the other showing complete infarction) at eighteen weeks.  $\times 26$ .

*C*, section of renal cortex from a rat deprived of 75 per cent of its renal tissue by extirpation. The same parenchymal changes are observed here as in *B*. Rat P-105 of group 4a (one kidney and half of the other extirpated) at thirty-four weeks.  $\times 26$ .

*D*, glomerulus from the same kidney as the section shown in *A*. The capillary loops are fused. The capsular space is obliterated, and there is thickening of the capillary and capsular membranes. Rat P-16 of group 4c (one kidney and half of the other showing complete infarction) at five weeks.  $\times 261$ .

*E*, glomerulus from the same kidney as the section shown in *B*. Note large foamy epithelial cells, fused capillary loops, thickening of Bowman's capsule and thickening of the wall of an afferent arteriole. Rat P-29 of group 4b (one kidney extirpated and half of the other showing complete infarction) at eighteen weeks.  $\times 261$ .

*F*, necrosis of the wall of an afferent arteriole with dilatation and thrombosis. Rat P-124 of group 4c (one kidney and half of the other showing complete infarction) at two weeks.  $\times 261$ .

of the rats had arterial lesions, 20 per cent showing alterations of a severe nature. The occurrence of vascular lesions did not correspond with the occurrence of high blood pressure. Since all but 1 of the animals survived for a year after operation, the vascular lesions found in this group were mostly healed or healing, although an occasional acute lesion was found as late as a year after infarction. There were

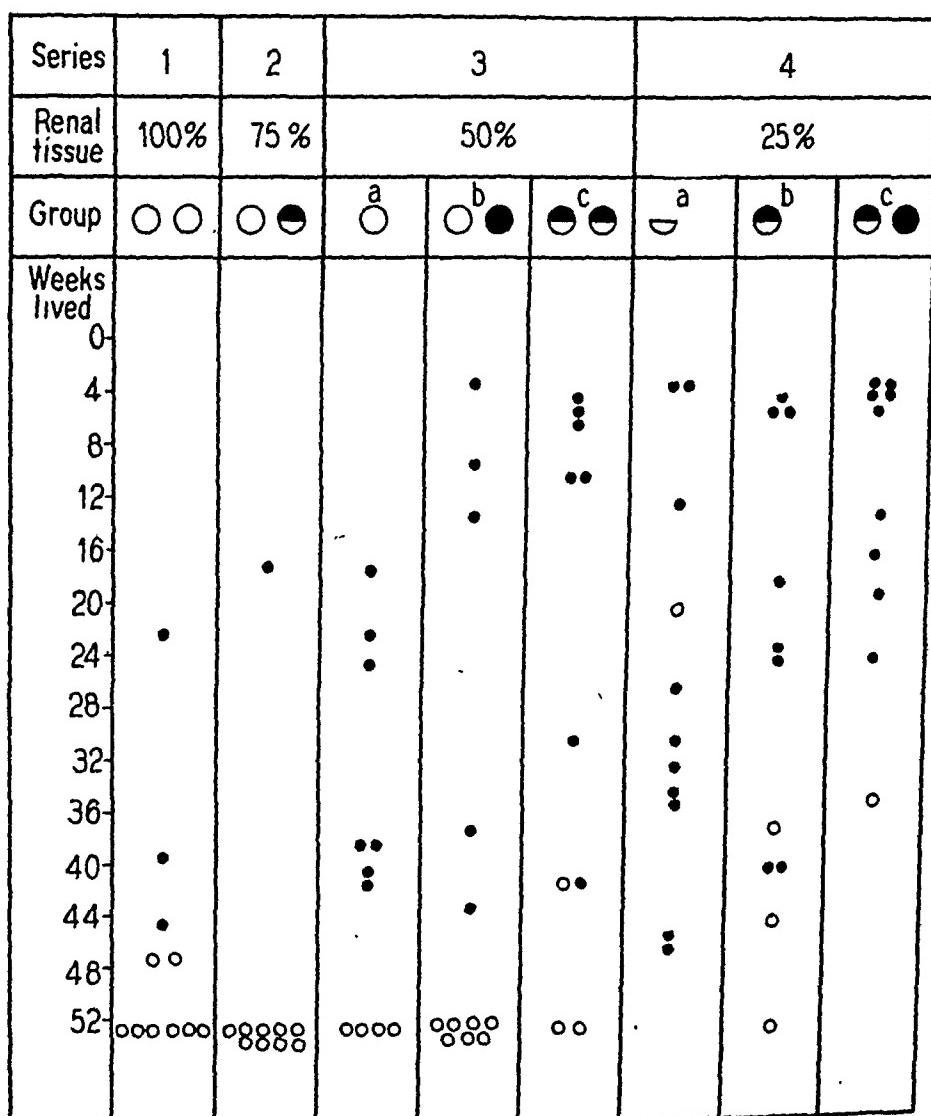


Fig. 7.—Chart showing time when death occurred in all rats living three weeks or longer after operation. Death by anesthesia ○; animal found dead •.

no changes in the intact renal tissue beyond moderate hypertrophy. One animal of the group, or 10 per cent, died before six months. Ninety per cent lived one year.

*Series 3* (animals with 50 per cent of their renal substance).—

(a) One kidney extirpated: In 2 of the 11 animals in this group the

blood pressure showed sporadic elevations reaching between 170 and 180 mm. of mercury (fig. 10 A), while in 2 others the blood pressure reached 160 mm. only once. The pressure curves of the remainder resembled those of the controls. All the individual and the mean curves are shown in fig. 11 A. None of these rats had arterial lesions. There was no alteration except hypertrophy in the remaining kidney. Twenty-seven per cent of the rats died before six months; 37 per cent lived one year.

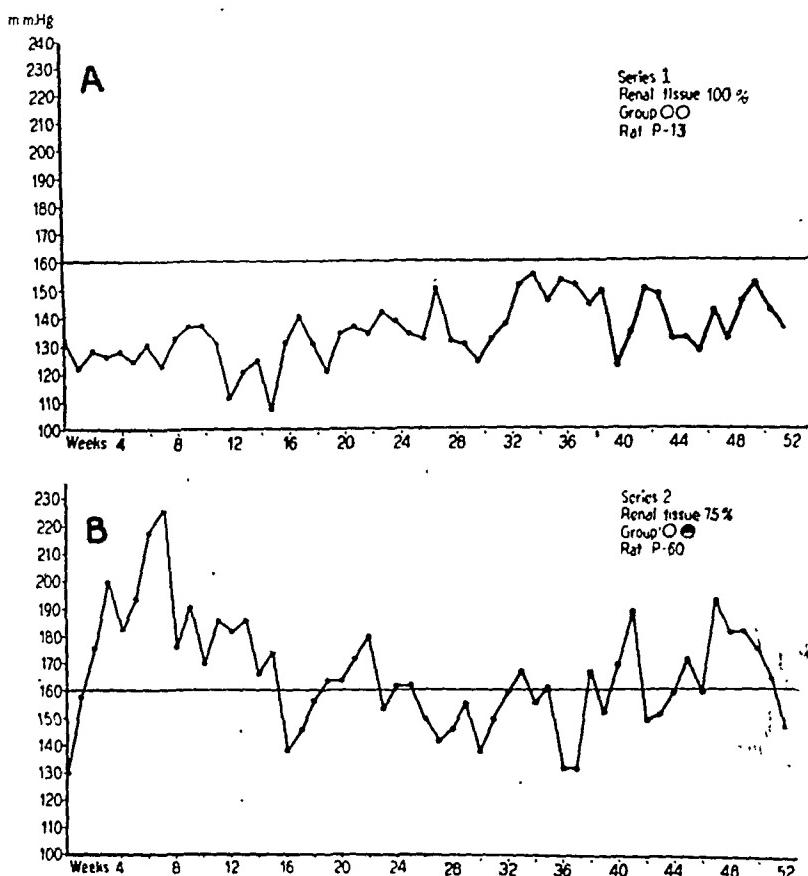


Fig. 8.—Blood pressure curves of individual rats in series 1 and 2. A, series 1 (control with both kidneys normal). B, series 2. Circles represent kidneys; black shading represents infarction.

(b) One entire kidney infarcted: The blood pressures in this group were variable. In 50 per cent of the animals the blood pressure was never above 160 mm. of mercury. Of the 6 animals which were hypertensive, 1 had a blood pressure of 160 mm. on one occasion, 1 died after hypertension of eleven weeks' duration, the pressure ranging around 170 mm. of mercury, and the blood pressure of the 4 remaining rats reached levels between 170 and 210 mm. and declined to a lower level during the latter part of the year (fig. 10 B). All the individual

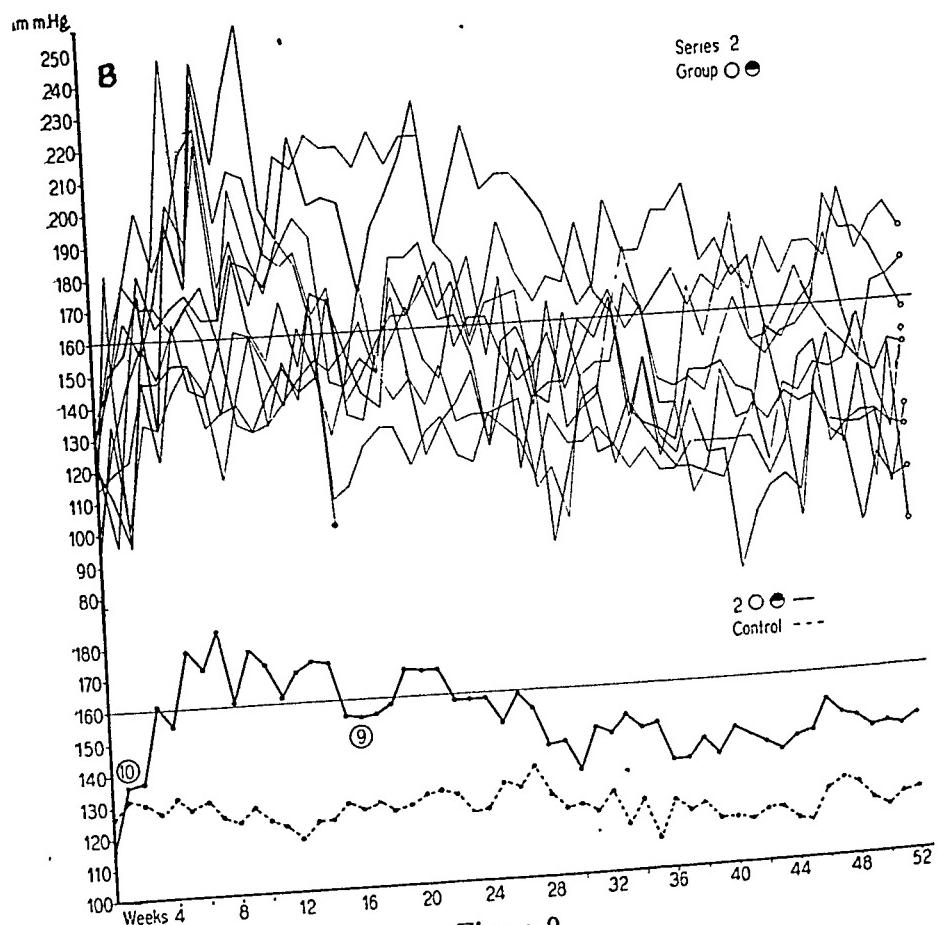
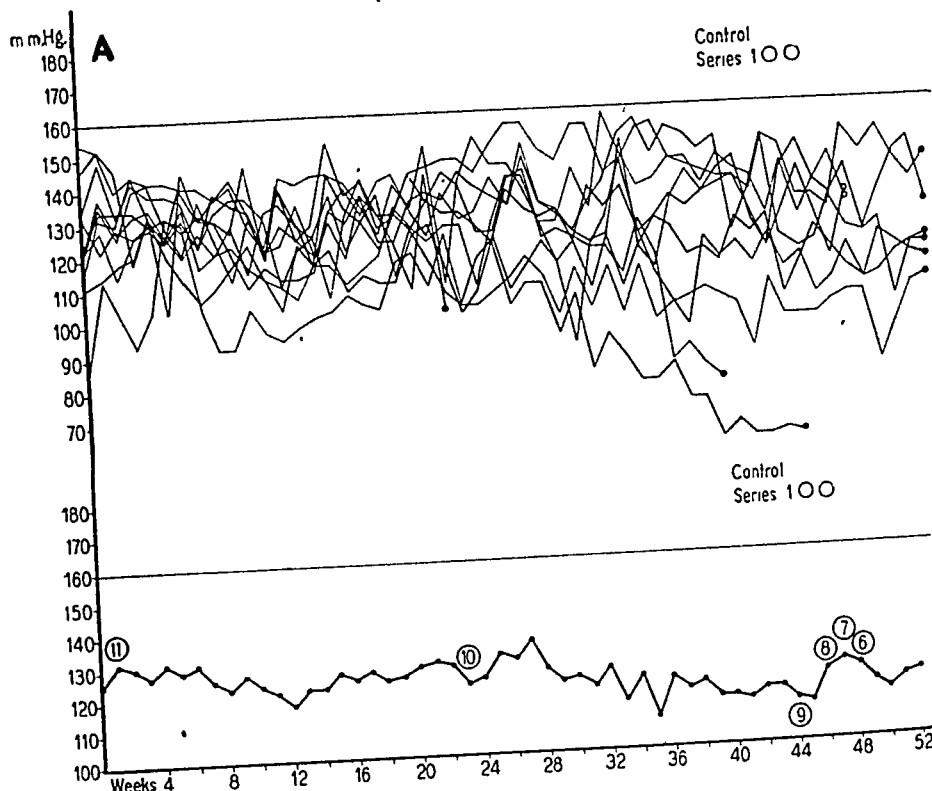


Figure 9

(See legend on opposite page)

and the mean curves are shown in figure 11 B. Arterial lesions occurred in 25 per cent and were moderately severe in only 1 animal. There was no correspondence between degree and duration of hypertension and occurrence of arterial lesions. The 1 animal in which moderately severe arterial lesions developed died at nine weeks without showing hypertension, while the 2 animals with hypertension above 200 did not show vascular lesions. There were no changes in the uninfarcted kidney except hypertrophy. Three, or 25 per cent, of the animals died before six months. Seven, or 68.3 per cent, lived one year.

(c) One half of each kidney infarcted: The blood pressure rose to about 160 mm. of mercury in all 10 rats in this group and above 200 in 40 per cent. The development of hypertension was prompt, occurring in 90 per cent of the rats in the first week, and by that time the blood pressure was 200 or above in 4 of the animals. The 5 animals surviving more than six months showed hypertension which diminished in later months. See figure 10 C. The curve of those dying earlier was similar in form to that of 13 C but the levels were not so high. All the individual and mean curves are shown in figure 12 A. The severity of the arterial lesions did not correspond to the degree of hypertension. There were no changes in the uninfarcted portions of the kidneys except hypertrophy. Fifty per cent of the animals died before six months. Twenty per cent survived one year.

*Series 4 (animals with 25 per cent of their renal substance).*—  
 (a) One kidney and half of the other extirpated: The blood pressure reached 160 mm. of mercury in 5 of the 11 animals, or in 45.4 per cent. No rats in this group had pressures above 200 mm. and only 1 above 180 mm. Hypertension did not develop in the first weeks after operation, the first affected animal showing elevated blood pressure at seven weeks. A typical curve in this group is represented by figure 13 A. Even in those animals whose blood pressures never rose to 160 mm. there was a tendency toward some elevation in the last months. All the individual and the mean curves are shown in figure 13 B. Mild vascular lesions were found in 81 per cent of the animals. Sometimes these consisted of a single nodule.

Fig. 9.—Blood pressure curves of all rats in control series 1 and series 2.

In this figure and in figures 11, 12, 14 and 15, the upper part of each chart shows individual curves; the lower part, the curve for the average of blood pressures. The symbol • ending a curve means that the animal died. The symbol O ending a curve means that the animal was killed. The encircled figures in each chart that shows average curves represent the number of animals in the experiment as it progressed. The average of the controls is shown by a broken line in all except figure 11 A. Circles represent kidneys; black shading represents infarction.

Besides hypertrophy, there were marked changes in the kidney remnant in 7, or 63 per cent, of the animals, while slight changes were observed in 2 and none in 2 animals that died at three weeks. Five,

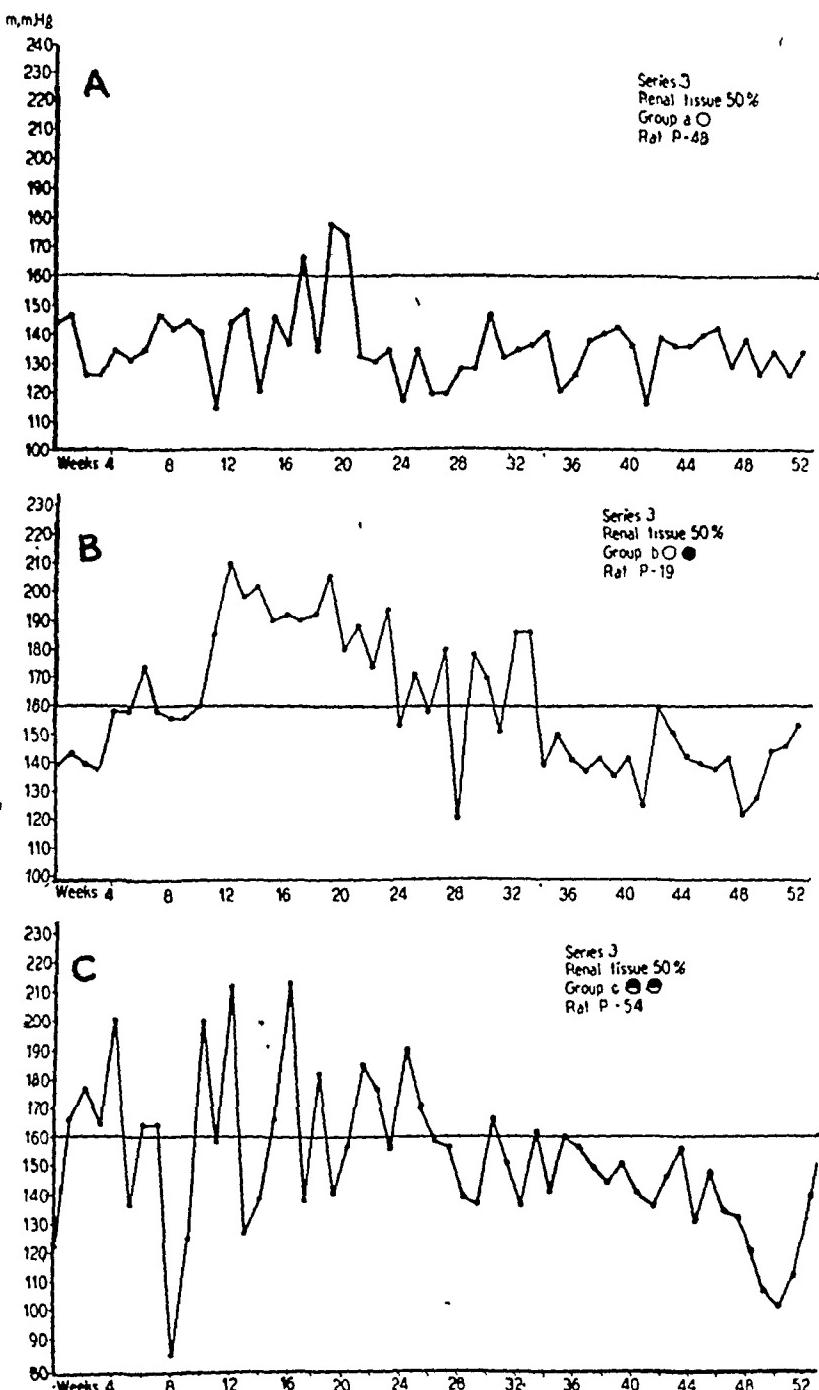


Fig. 10.—Blood pressure curves of individual rats in series 3. A, B and C, series 3, groups a, b and c, respectively.

or 45 per cent, died before six months. None survived for one year. The 2 rats living the longest died in the forty-sixth week.

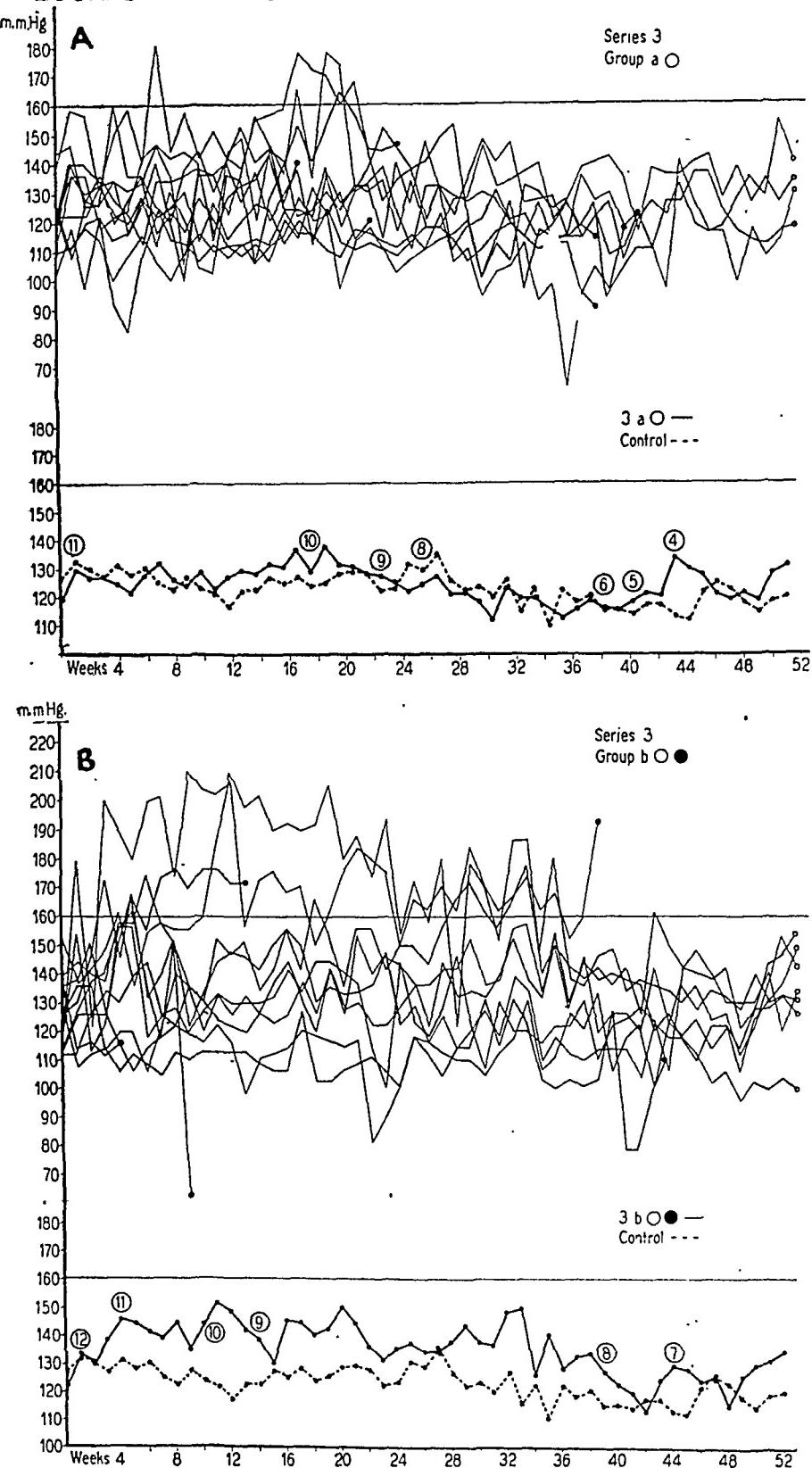


Fig. 11.—Blood pressure curves of all rats in group *a* and group *b* of series 3.  
(See the legend of figure 9 for further explanation.)

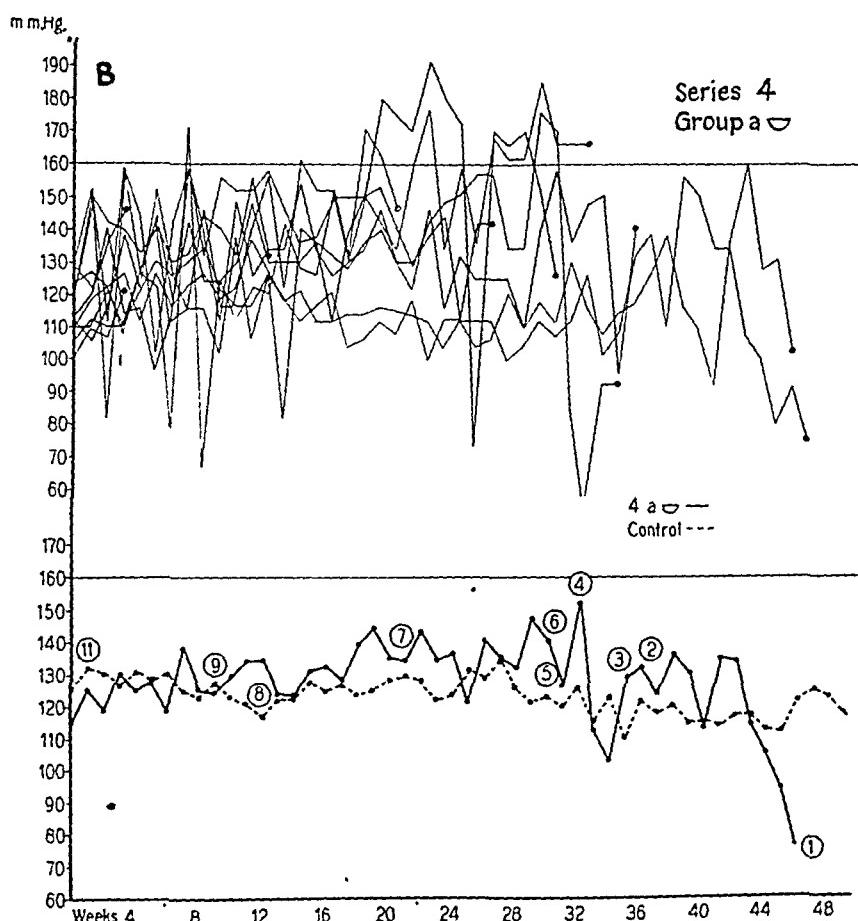
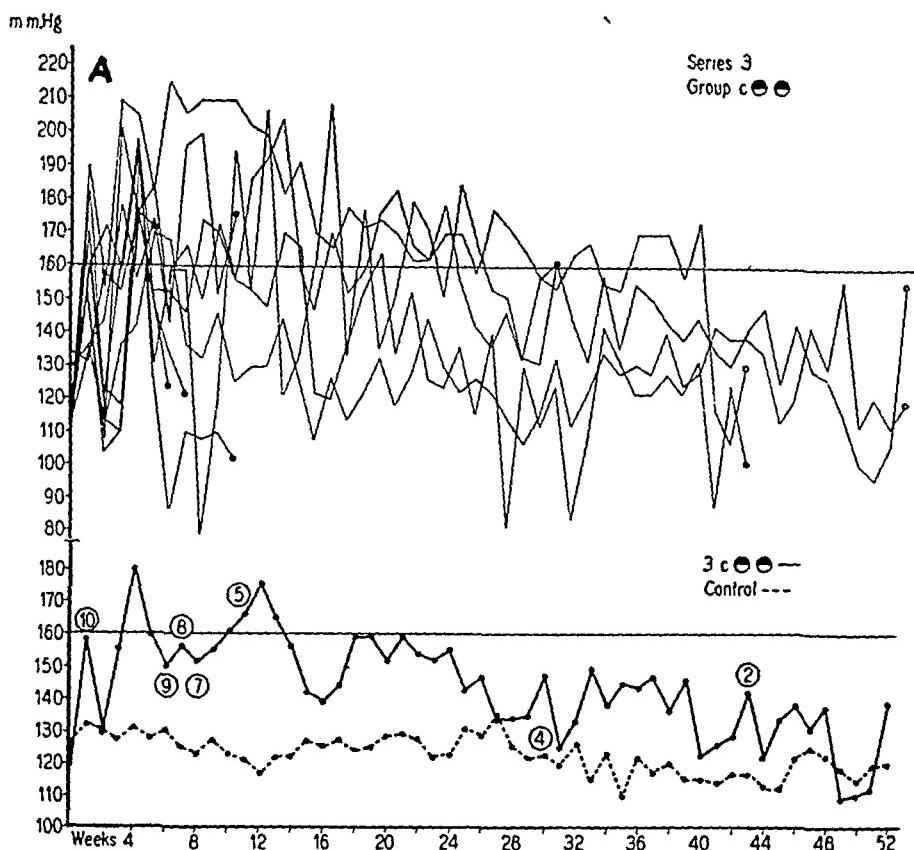


Fig. 12.—Blood pressure curves of all rats in series 3, group c, and series 4, group a. (See the legend of figure 9 for further explanation.)

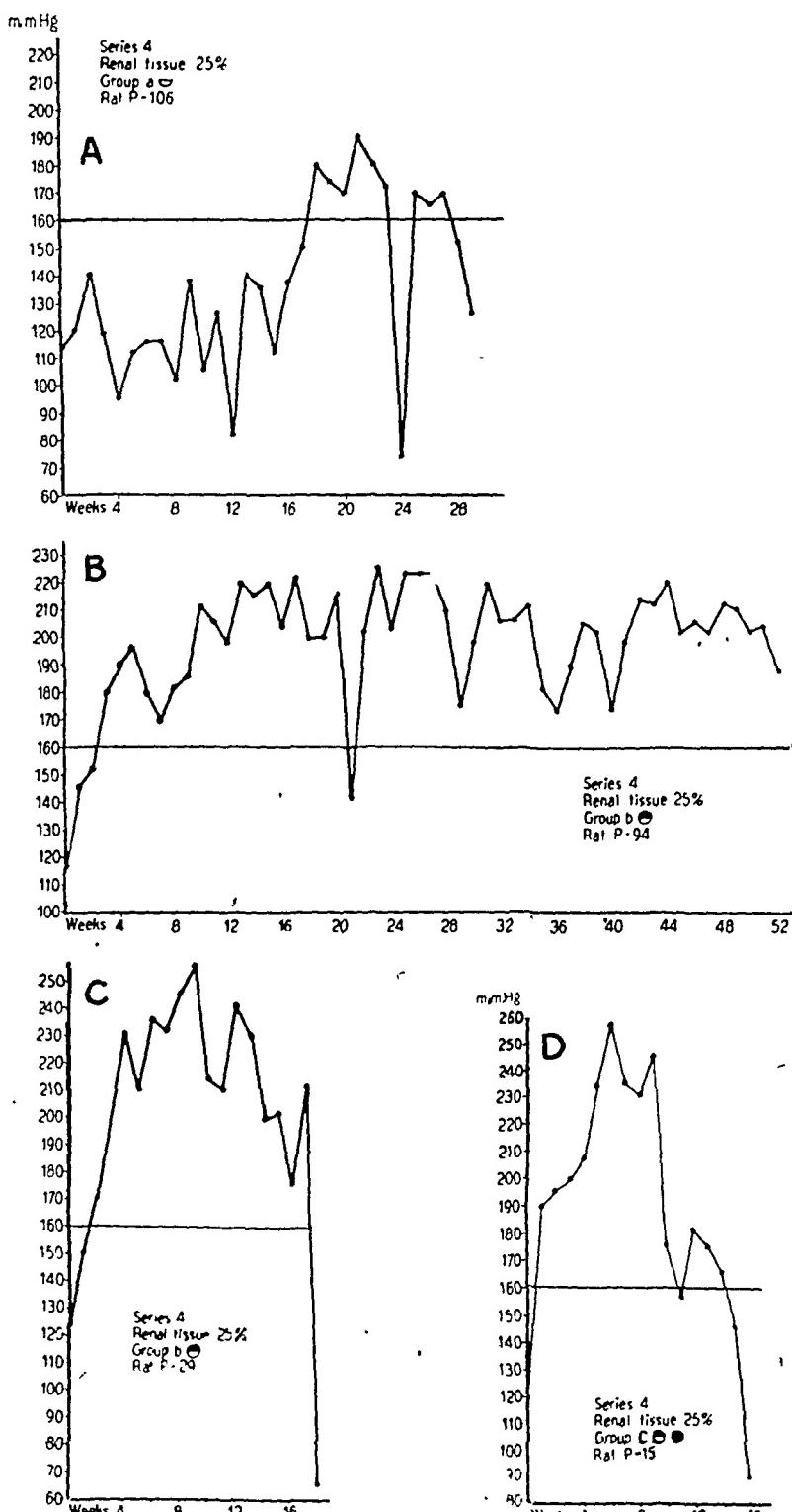


Fig. 13.—Blood pressure curves of individual rats in series 4. *A, B, C* and *D*, series 4, groups *a*, *b* (represented by 2 rats) and *c*, respectively.

(b) One kidney extirpated; one half of the other infarcted: Ninety per cent of these rats showed blood pressures above 160 mm. of mercury. One animal dying in the fifth week revealed no hypertension. Seventy-two per cent of them had pressures above 200 mm. The hypertension developed early, and the blood pressure rose steeply. The high level

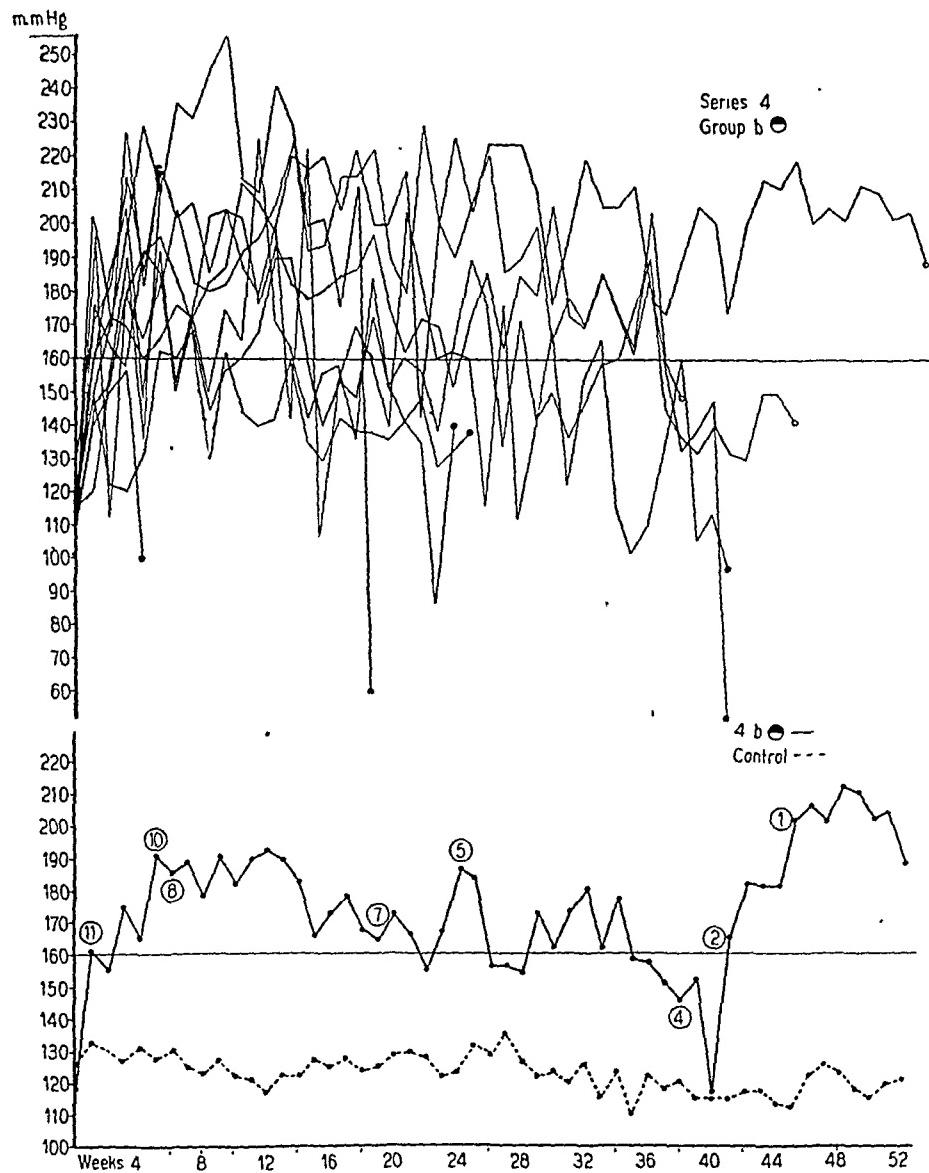


Fig. 14.—Blood pressure curves of all rats in series 4, group b. (See the legend of figure 9 for further explanation.)

of hypertension was maintained in all hypertensive rats of this group except 1 whose curve resembles that of figure 10 B. Figure 13 B shows the weekly pressures of a rat throughout one year, while figure 13 C shows those of a rat dying at eighteen weeks. All the individual and the mean curves are shown in figure 14. Arterial lesions were found

in all of these animals and in 54 per cent of them were severe and extensive. Besides hypertrophy, renal changes of the type shown in figure 6 C were found in all of the group except the rat which died in the fifth week. Fifty-four per cent of the 11 rats in this group died before six months. One animal survived one year.

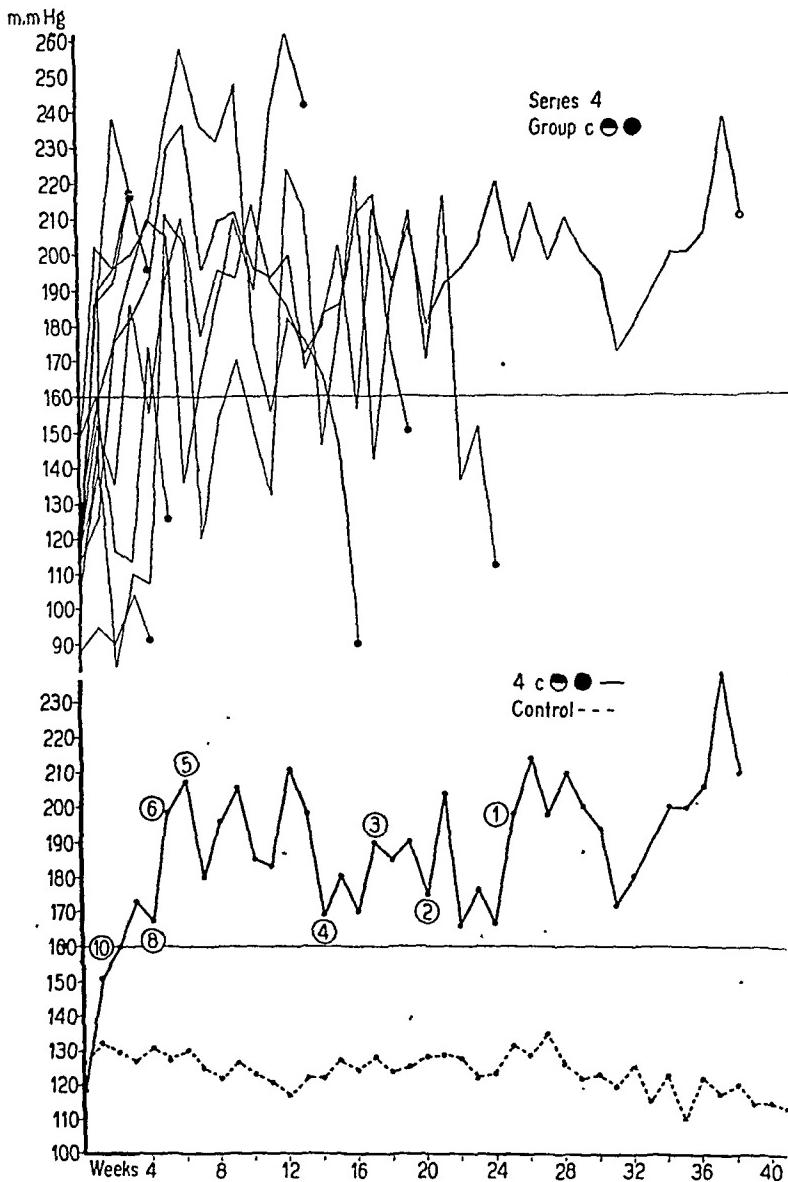


Fig. 15.—Blood pressure curves of all rats in series 4, group c. (See the legend of figure 9 for further explanation.)

(c) One entire kidney and one half of the other infarcted: In this group the blood pressure rose above 160 mm. of mercury in 90 per cent of the animals and above 200 in 80 per cent. One animal showing no hypertension died in the fifth week. Elevation of pressure appeared

in 70 per cent of the animals in the first week. The blood pressure curves were similar to those of group *b*. (In fig. 13 compare *D* with *C* and *B*). All the individual and the mean curves are shown in figure 15. The 1 rat which lived more than six months had sustained hypertension until death at thirty-eight weeks. The arterial lesions in this group were severe and occurred in 100 per cent of the animals. Seventy per cent of these rats had extreme arterial involvement. Changes besides hypertrophy were found in the intact renal tissue of all animals but were not marked in those dying early. Ninety per cent of this group died before six months. None survived a year.

The foregoing data are summarized in figure 16.

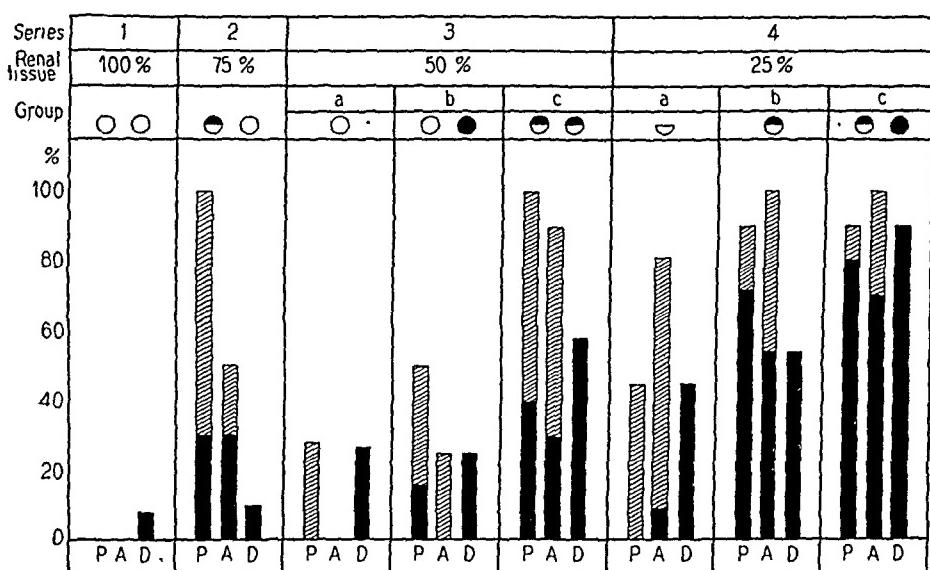


Fig. 16.—A comparison of the effects of various amounts of renal infarction and extirpation. *P* signifies blood pressure; *A*, arteritis; *D*, deaths before twenty-six weeks.

In regard to blood pressure, the cross-hatched column represents rats with blood pressure of 160 mm. of mercury or higher and the solid black column rats with blood pressure of 200 mm. of mercury or higher.

In regard to arteritis, the cross-hatched column represents rats with any type of arteritis and the solid black column rats with arteritis of severe type.

## COMMENT

The first question posed at the beginning of this paper was whether or not renal infarction causes hypertension. To a certain extent this had been affirmatively answered by the experimental and clinical observations of a number of workers in the past. Goldblatt<sup>13</sup> summarized the experimental work in this and related fields in 1938. Much of

13. Goldblatt, H., in The Harvey Lectures, 1937-1938, Baltimore, Williams & Wilkins Company, 1938, vol. 33, p. 237.

the early experimental work, of which that done by Cash<sup>14</sup> is the most extended and significant, is confused and inconclusive and has given rise to unfounded conceptions and simplifications, which have in turn influenced later studies. One of these, for example, is that hypertension following infarction, unless it occurs immediately, does not develop at all. Another is that a large amount of tissue must be infarcted for hypertension to occur. Both these concepts have been found by me not to be true of the rat. It is hoped that the results of the experiments reported here clarify to some extent the details of the problem.

In the clinical field the association of renal infarction and hypertension has been frequently noted, but the opinion that they are causally related has been voiced in few instances, a notable exception being the report of a case studied by Prinzmetal, Hiatt and Trageman.<sup>15</sup> Fishberg<sup>16</sup> and Yuille<sup>17</sup> have reported similar cases. The well known case of Boyd and Lewis<sup>18</sup> has been dismissed from consideration as an instance of hypertension due to infarction by some commentators<sup>19</sup> and indeed by the authors of the report themselves, because they thought that changes in renal arterioles might have accounted for the elevation of blood pressure.<sup>20</sup> Yuille<sup>17</sup> has recently reviewed the clinical aspect of these questions in a consideration of the relation of obstructive lesions of the main renal artery to hypertension.

In the present status of the problem, uncertain both from the experimental and from the clinical side, it was felt that a reexamination of this questioned point with the rat as the experimental animal was desirable and timely especially since I had gained a background of anatomic facts from a detailed exploration of infarcted renal tissue by newer methods.<sup>1</sup>

The results of the experimental work reported here establish that renal infarction is a potential cause of hypertension in rats since it elicits this response if the development of hypertension is not hindered by other factors. The same statement may be made in regard to the part played by infarction in the production of vascular lesions of a necrotizing and inflammatory type.

14. Cash, J. R.: Bull. Johns Hopkins Hosp. **35**:168, 1924; Proc. Soc. Exper. Biol. & Med. **23**:609, 1926.

15. Prinzmetal, M.; Hiatt, N., and Trageman, L. J.: J. A. M. A. **118**:44, 1942.

16. Fishberg, A. M.: J. A. M. A. **119**:551, 1942.

17. Yuille, C. L.: Am. J. M. Sc. **207**:394, 1943.

18. Boyd, C. H., and Lewis, L. G.: J. Urol. **39**:627, 1938.

19. Smith, H. W.; Goldring, W., and Chassis, H.: Bull. New York Acad. Med. **19**:449, 1943.

20. If arteriolar changes had been the mechanism in this case, it is unlikely that such alterations would have been unilateral, so that the drop in pressure which occurred on removal of the infarcted kidney would in fact be inexplicable according to their interpretation.

The acute experiments of Winternitz and co-workers,<sup>21a</sup> using the dog, constitute the only work in which there has been a study of these relationships. These workers demonstrated that necrosis of vascular walls occurred after ligation of both renal arteries. They were able to elicit only minimal lesions when the artery to only one kidney was ligated.<sup>21b</sup> In my experiments it has been shown that in the rat necrotizing and inflammatory changes of vascular walls followed infarction of the kidney and occurred not only when there was infarction of considerable tissue but also when 75 per cent of the normal tissue remained intact and there could be no question of renal insufficiency.

Having established by the present investigation that in the rat renal infarction is regularly followed by physiologic and anatomic changes in the vascular system, the query as to whether these effects can be explained by absorption from the infarct can be examined. Certain experiments besides those of Winternitz and his group, just cited, suggest that they can be so explained. For example there are those of Taquini<sup>22</sup> with the dog, and those of Prinzmetal and his collaborators<sup>23</sup> with several species of animals, including the rat, in which the renal artery was completely occluded by a clamp for a period of several hours and in which on release of the clamp elevation of blood pressure occurred. There is also Goldblatt's<sup>24</sup> experiment in the dog in which hypertension failed to follow occlusion of both vein and artery but developed when the artery alone was occluded. Prinzmetal and co-workers<sup>25</sup> observed that in cats extracts of kidneys which had been deprived of their circulation for six hours and the perfusate of a kidney of a human subject<sup>15</sup> with renal embolism yielded a greater amount of potential pressor substance than was found in normal kidneys. In all these experiments there is certainly evidence that can be taken to mean that blood flowing through renal tissue after complete ischemia carried out a substance which acted to elevate blood pressure. If this is so why then is there not hypertension in all cases of renal infarction and why does not the rise in pressure occur shortly after occlusion of the renal vessel?

The reaction of hypertension under these conditions appears to depend on the fulfilment of several requirements, the most obvious of

21. (a) Winternitz, M. C.; Mylon, E.; Waters, L. L., and Katzenstein, R.: Yale J. Biol. & Med. **12**:623, 1940. (b) Winternitz, M. C., and Katzenstein, R.: ibid. **13**:15, 1940.

22. Taquini, A. C.: Rev. Soc. argent. de biol. **14**:222, 1938.

23. Lewis, H. A.; Leo, S. D., and Prinzmetal, M.: Am. Heart J. **21**:319, 1941.

24. Goldblatt, H.: Ann. Int. Med. **11**:69, 1937.

25. Leo, S. D.; Prinzmetal, M., and Lewis, H. A.: Am. J. Physiol. **131**:18, 1940.

these being that the pressor substance must gain entrance into the blood stream. If it is present in the injured tissue, in order for enough of it to be absorbed into the blood to be expressed in terms of hypertension, blood needs to flow through the infarct to a sufficient extent that drainage of the injured area is possible. In the Taquini and Prinzmetal experiments cited, such a flow was accomplished simply by removing the clamp from the artery. When, however, a renal artery is ligated, or completely occluded by a thrombus as in human cases, the blood can flow into the infarct from the neighboring renal parenchyma only by way of the narrow intrarenal anastomotic connections of the intertubular network or through pathways even less direct and numerous than these, namely, the extrarenal capillary anastomoses, and against the impediments offered by stasis, edema and clotting. A lapse of time is therefore necessary before the flow is established through a sufficient mass so that the absorption of effective amounts of the hypertensive agent may occur.

If hypertension depends on absorption of the necrotic tissue, it is less likely to develop at all, or it should take longer to do so, in cases in which the establishment of contact between the necrotic tissue and the circulating blood is delayed than in those in which it is more immediate. This is demonstrated in the experiment in which one main renal artery was ligated and severed (group 3 b, one kidney showing complete infarction), for in half of these rats hypertension did not develop. In the animals of this group which did exhibit hypertension it developed more slowly than in any other group of rats with infarcted tissue. The mass of necrotic tissue was, however, equal to or more than that in other groups in which hypertension appeared more rapidly and in 100 per cent of the animals (group 2, with infarction of one half of one kidney, and group 3 c, with infarction of one half of each kidney). The obvious difference between these groups is that in group 3 b there were not abundant intrarenal anastomoses between the vessels of the injured tissue and the general circulation by which blood could enter directly into the peripheral part of the infarct from the neighboring renal parenchyma. It could, indeed, come into the necrotic mass only by the extrarenal anastomoses between the branches of the renal artery which supply the perirenal fat, the capsule, the ureter and the pelvis; arising proximal and distal to the point of section, and which anastomose outside of and at some distance from the necrotic tissue. The time required for the circulation to be established in the necrotic mass must necessarily, therefore, have been greater in rats with complete infarction of one kidney than in rats with partial infarction of both kidneys. No other explanation seems possible than that the difference in hypertensive reaction is based on the anatomic factors

detailed in the foregoing paragraph, and this leads to the conclusion that the hypertension which follows infarction is due to absorption.<sup>26</sup>

The duration of hypertension also appears to be related to absorption. During the period of revascularization of the infarct, the contact of the necrotic tissue with the blood is increasing, yet the mass of necrotic material is decreasing, so that though an increasingly greater flow is established throughout the tissue, with the passage of time there is progressively less substance to be drained. In an earlier study<sup>1</sup> it was found by dissection that the extension of patent blood vessels throughout the infarct ceased in the second month after infarction. Loss of substance in the infarct, however, continued for an indefinitely longer period. The hypertension, if it is an effect of absorption, should therefore diminish after an interval, and this is what happened in the groups of animals with more than 25 per cent of the renal tissue uninvolved by infarction. The blood pressure declined to considerably lower levels after a period of from three to six months, and sometimes reached a normal figure. Though in some of these rats hypertension persisted until the end of the experiment, in none was the initial high level maintained. The mechanism by which hypertension, even if at lower levels, is thus prolonged after the tissue might presumably be exhausted of its pressor substance will be discussed later.

Can the necrosis and inflammation of the arteries be explained by the assumption that they, too, are due to absorption of products from the infarct? I believe that they can at least partially be explained along the lines of the preceding argument, in spite of the fact that they occur also when a large amount of renal tissue is removed but the kidney is not otherwise injured. The evidence that they are related to absorption from the infarct is found in the fact that in the animals with infarction vascular arterial lesions were exhibited most extensively and most severely when absorption was occurring most rapidly as evidenced by the rapidity of the reduction in size of the necrotic mass. Animals dying in the last twenty-six weeks of the experiment showed in the main no vascular lesions or none that were acute. It was also observed that the group which had structurally the least efficient means for drain-

26. The fact that one rat in each of the groups 4b and 4c did not show hypertension in the four weeks which they lived after infarction does not invalidate this conclusion. These two animals were in poor condition from the time of operation and did not make a proper recovery. Both, however, showed arterial lesions. That the ability to react to the stimulus which raises blood pressure is lacking in sick animals is well recognized clinically, and this weakness may account, according to Fishberg,<sup>16</sup> for the irregularity of hypertension following renal embolism in human beings and for the fall in blood pressure which sometimes occurs in the final days before death in formerly hypertensive persons. The latter is a phenomenon which was often exhibited in these experiments.

age of the infarct, i. e., group 3 b, discussed in a foregoing paragraph, showed the fewest and mildest arterial lesions.

As opposed to the factor of absorption, the part played by the reduction of renal tissue in the effects observed after renal infarction must next be considered. Winternitz and associates in their studies<sup>21a</sup> observed that necrotizing vascular lesions occurred in the dog after complete removal of the renal tissue, i. e., bilateral nephrectomy, but that they were much more numerous and severe when both kidneys showed complete renal infarction or when both ureters were ligated. Holman<sup>27</sup> also observed in the dog that vascular lesions occurred following total nephrectomy alone.

It was demonstrated in my experiments that the reduction of the renal tissue to 50 per cent of its original mass does not produce hypertension or arterial lesions while the loss of 75 per cent may result in both these conditions. The vascular injury in the latter instance was, however, in most of the rats minimal, and to locate it required meticulous observation.

As to the hypertension, reduction of renal tissue has been found to produce it in the rat by a number of workers, notably Chanutin and Ferris,<sup>28</sup> Rytand and Dock<sup>29</sup> and Wood and Etheridge.<sup>30</sup> It is difficult or impossible, on the other hand, to cause elevation of blood pressure by this means in the dog.<sup>31</sup> In the experiments reported here, reduction of renal substance to 25 per cent resulted in hypertension after several months in some of the animals. The difference in degree, however, between the hypertension appearing after infarction and that after subtotal nephrectomy indicates that they must be due to different initial mechanisms, for there can be no question of absorption in the latter case.

It is clear, however, that an influence must be ascribed to the factor of loss of renal tissue when the removal of a large amount of tissue (50 per cent) is combined with infarction; for severe arterial lesions, blood pressures persisting at high levels (over 200) and early death of the animals did not result from either procedure alone but from their combination.

The mechanism operating from loss of renal tissue appears to be one by which the activity of an agent affecting the vasculature is enhanced in the milieu created by reduction of normal renal function below a certain critical level. It seems apparent from this, as has been pointed

27. Holman, R. J.: Am. J. Path. **19**:159, 1943.

28. Chanutin, A., and Ferris, E. B.: Arch. Int. Med. **49**:73, 1932.

29. Rytand, D. A., and Dock, W.: Arch. Int. Med. **56**:511, 1935.

30. Wood, E. J., and Etheridge, C.: Proc. Soc. Exper. Biol. & Med. **30**:1039, 1933.

31. Mason, M. F.; Robinson, C. S., and Blalock, A. J.: J. Exper. Med. **72**:289, 1940.

out in discussions of the humoral mechanism of renal hypertension,<sup>32</sup> that normal renal tissue can neutralize, modify, destroy or excrete those substances which bring about the vascular changes. The balance of opinion, as far as hypertension is concerned, is that normal renal tissue neutralizes or destroys the pressor substance and does not excrete it. This has been demonstrated in the work of Rodbard and Katz.<sup>33</sup> Certain agents, if present in sufficient quantity in the internal environment created when the amount of normal renal tissue is insufficient for their neutralization, are capable of eliciting the vascular phenomena. These substances may be formed in necrotic renal tissue or in renal tissue functionally deranged and, in the case of total nephrectomy, in the absence of renal tissue.

It is difficult in any particular instance to determine, therefore, which factor, infarction of renal tissue or insufficiency of renal tissue, is of the greater relative importance in the production of the vascular effects. However, in analyzing the results in groups 2, 3c and 4b (fig. 16) it is quite evident that the amount of normal renal tissue remaining is more important in determining the degree of anatomic or physiologic change in the vessels than the amount of tissue showing infarction, for with decreasing amounts of renal tissue the disturbances become progressively more pronounced.

It was observed not only that the excision of renal tissue enhanced the effects of infarction on the vascular system but that progressive degenerative changes took place in the remaining renal tissue when the reduction was as high as 75 per cent. These renal changes appear to be of the same sort and degree as those described by some<sup>34</sup> as resulting from subtotal nephrectomy and those found in the dietary experiments of others,<sup>35</sup> in most of which at least one kidney had been removed. The resulting degenerative changes in the glomeruli and the tubules appear to be the result of overloading of the kidney remnant and production of functional strain, as suggested by Wood and Etheridge.<sup>36</sup> Whatever their origin they still further reduce the amount of functioning renal tissue, so that an acceleration of the complete cycle occurs.

32. Lewis, H. A., and Goldblatt, H.: Bull. New York Acad. Med. **18**:459, 1942. Grollman, A.; Harrison, T. R., and Williams, J. R., in Publication 13, American Association for the Advancement of Science, 1940, p. 274.

33. Rodbard, S., and Katz, L. M.: J. Exper. Med. **73**:357, 1941.

34. Gross, P.; Cooper, F. B., and Morningstar, W. A.: Am. J. Path. **13**:101, 1942. Wood and Etheridge.<sup>36</sup> Pappenheimer, A. M.: J. Exper. Med. **64**:965, 1936.

35. Allen, R. B.; Bollman, J. L., and Mann, F. C.: Arch. Path. **19**:341, 1935. Chanutin, A., and Ludewig, S.: Arch. Int. Med. **58**:60, 1936. Moise, T. S., and Smith, A. H.: Arch. Path. **4**:530, 1927. Newburg, L. H., and Curtis, A. C.: Arch. Int. Med. **42**:801, 1928. Medlar, E. M., and Blatherwick, N. E.: Am. J. Path. **13**:881, 1937.

Another factor contributing to the disturbance of renal function and hypertension may be the renal arterial lesions which, while minimal or absent in most animals in my experiments, were quite extensive in those groups with infarction plus 75 per cent reduction of normal renal tissue.

On the basis of the foregoing considerations, the factors which may interact to effect and enhance the vascular reactions of hypertension and the arterial lesions in these experiments may be summarized as follows:

*I. Factors Related to Infarction (Necrosis of Renal Tissue):* 1. The extent of the infarct (amount of pressor substance). 2. The degree of revascularization of the necrotic tissue (facility of absorption).

*II. Factors Related to the Amount of Renal Tissue Remaining:* 3. The degree to which reduction of renal tissue diminishes an agent derived from the living tissue that neutralizes the pressor substance of the infarcted tissue. 4. The degree to which the metabolic overload of the remaining tissue reduces renal function. 5. The extent to which renal vascular lesions progressively reduce renal tissue and function. 6. A factor causing hypertension and vascular lesions which is present as a direct result of excessive reduction of renal substance (75 per cent nephrectomy).

In table 2 each of these hypothetic factors is given a value as it may be estimated from the conditions of the experiments and microscopic evidence. These values are totaled for each experimental group, and the observed results of the experiments are ranged beside them for comparison.

Although there are some discrepancies and the whole structure is suppositious, the order of the assigned values agrees fairly well with the order of the magnitudes of the observed results.

The observations made in these experiments seem, therefore, to point to the conclusion that necrotic renal tissue is a source and reservoir for an agent, or agents, which when absorbed into the blood can cause hypertension and necrotizing inflammatory lesions of the arteries. It may only be conjectured whether there are a number of agents effecting these conditions, whether the action is direct or mediated and whether the agents are the same as the humoral elements which are responsible for the results obtained when the hemodynamics of living renal tissue are altered by constricting the renal artery.

One objection that might be raised to this hypothesis is that the hypertension from infarction in these experiments was too protracted to be due to a substance lasting for so long a time in necrotic tissue, and the opinion might be held that some further added mechanism is implicated which would bring about a renewal of the effective substance. Such a mechanism might be a reduction due to the restrictive effect of the scar of the infarct on the pulse pressure in the unaffected portion of the kidney, especially the tissue which survives at the border of the infarct. This

explanation might be valid were it not for those experiments in which hypertension developed following complete infarction of the whole kidney, in which there was no living tissue. Moreover, there is scar tissue at the site of the removal of the poles in three-quarter nephrectomized animals without renal infarction, but the effects were different in time of development and in degree from those observed after infarction, as has been shown.

TABLE 2.—*A Comparison Between the Numerical Evaluation of Hypothetic Factors in the Condition of Each Group and the Observed Results*

Group	Hypothetic Factors *						Total Numerical Value*	Results				
	1	2	3	4	5	6		Blood Pressure Above:	Arterial Lesions	Dead Before 26 Weeks		
	Extent of Infarct	Blood Supply to Infarct	Loss of Renal Tissue with Consequent Loss of Neutralization Factor	Loss of Renal Function Due to Damage of Remaining Tissue by Overload	Loss of Renal Tissue from Vascular Damage	Direct Factor Present as a Result of 75% Reduction of Renal Substance with No Infarct		160 Mm.Hg	200 Mm.Hg.	Any Type	Severe Type	
4c e	+++	++888	+++	++	++	+	16	90	80	100	70	90
4b e	+	++8	+++	++	++	+	12	90	72	100	54	54
3c e e	++	++8++8	++	0	+	0	11	100	40	90	30	60
2 e o	+	++8	+	0	+	0	6	100	30	50	30	10
3b e o	++	88	++	0	0	0	6	50	16	25	0	25
4a e	0	0	0	++	+	+	4	45	0	81	9	45
3a o	0	0	0	0	0	0	0	27	0	0	0	27
1 o o	0	0	0	0	0	0	0	0	0	0	0	8

\*Each + is assigned a numerical value of 1

Column 1 - amount of necrotic renal tissue; Each + represents infarction of one half of one kidney.  
 Column 2 - blood supply to infarct; ++ signifies a supply from peripheral uninjured tissue;  
 ++ signifies a supply from extrarenal connections.  
 Column 3 - loss of renal tissue and therefore of neutralization factor; Each + represents reduction of neutralization of products of infarction by loss of half of one kidney.  
 Column 4 - degenerative changes in remnant of kidney according to microscopic evidence:  
 ++ severe; + mild; 0 none.  
 Column 5 - changes in remnant of kidney due to vascular damage according to microscopic evidence: ++ marked, + minimal, 0 none.  
 Column 6 - influence of 75 per cent reduction of renal substance alone.

The persistence of renal hypertension after the removal of its apparent source has been observed and discussed by several workers. Kempf and Page<sup>4</sup> stated that hypertension caused by cellophane peri-nephritis of one kidney can be made to disappear by removal of that kidney if the condition has not been of too long duration. Wilson and Byrom<sup>7</sup> also found persistence of hypertension after removal of the kidney which had had its artery constricted. They explained this persistence by vascular changes occurring in the other kidney as a result of the "vascular crises" of hypertensive animals. Friedman, Jarmon and Klemperer,<sup>8</sup> similarly observed that hypertension remained after the treated kidney was removed and, believing that vascular lesions in the other kidney did not fully account for this persistence, suggested the possible participation of some extrarenal mechanism.

Reed, Saperstein, Southland and Ogden<sup>36</sup> proposed a nervous mechanism.

A possible explanation for the persistence of the hypertension after the supply of pressor substance in the necrotic mass might presumably be exhausted is that an initial substance, absorbed from the infarct, so sensitizes the vessels that the response to other or similar substances, entering the blood in minute amounts as a result of normal metabolism, becomes greatly exaggerated. This idea has been explored by Page<sup>37</sup> as regards renin and angiotonin. He observed that hypertensive animals were slightly sensitive to renin but not to angiotonin and concluded that in such animals there is an increase in renin activator and not hypersensitivity of the blood vessels.

The histologic appearance of the vascular lesions seen in the present study suggests a sensitizing mechanism of a hyperergic nature. The lesions are histologically identical with those produced in rats and rabbits with such substances as antikidney serum,<sup>38</sup> bacterial vaccines,<sup>39</sup> horse serum<sup>40</sup> and pig renin<sup>41</sup> and are also similar to the lesions observed by Clark and Kaplan<sup>42</sup> and by Rich<sup>43</sup> in patients to whom serum and sulfonamide compounds had been administered. Certainly these lesions, appearing as they do in the absence of hypertension, cannot be explained as due to the fluctuation between high and low pressures as proposed by Wilson and Byrom.<sup>7</sup> They are also found in the absence of renal failure, which Goldblatt<sup>44</sup> held was a necessary condition to their production.

And finally, it must also be kept in mind that there may be one unifying underlying principle at the root of the similar vascular reactions which appear either in response to a wide variety of experimental procedures or naturally.<sup>45</sup> As suggested by Selye and Pentz,<sup>46</sup> there may be participation of the adrenal cortical hormone or there may be some general colloid disturbance such as Hueper<sup>45b</sup> has

36. Reed, R. K.; Saperstein, L. A.; Southland, F. D., and Ogden, E.: Am. J. Physiol. **141**:709, 1944.

37. Page, I. H.: Am. J. Physiol. **134**:789, 1941.

38. Masugi, M., and Sato, Y.: Virchows Arch. f. path. Anat. **293**:615, 1934.

39. Metz, W.: Beitr. z. path. Anat. u. z. allg. Path. **88**:18, 1936.

40. Rich, T., and Gregory, J. E.: Bull. Johns Hopkins Hosp. **72**:65, 1943.

41. Leiter, L., and Eichelberger, L.: J. Clin. Investigation **22**:11, 1943.

42. Clark, E., and Kaplan, B. I.: Arch. Path. **24**:458, 1937.

43. Rich, T.: Bull. Johns Hopkins Hosp. **71**:123, 1942.

44. Goldblatt, H.: J. Exper. Med. **67**:809, 1938.

45. (a) Rackemann, F. M.: Arch. Int. Med. **73**:248, 1944. (b) Hueper, W. C.: Arch. Path. **38**:162, 245 and 350, 1944; **39**:51, 117 and 187, 1945. (c) Smith and others.<sup>9e</sup>

46. Selye, H., and Pentz, E. I.: Canad. M. A. J. **49**:264, 1943.

held responsible for inflammatory vascular reactions with fibrinoid necrosis. Such a "plasmatic" colloid disturbance related to the degree of dispersion of interacting colloid particles might arise in the allergic state. The puzzling fact that so many investigators have produced similar results by such diverse means seems to lead to the conclusion that a multiplicity of measures results in identical necrotizing and inflammatory lesions. This suggests that the underlying mechanism must be a basic change occurring when the parts of a constellation fall into place, a constellation whose structure is such that in one of its parts diverse entities can substitute one for another without altering the effective pattern. It is obvious that having reached this stage of speculative hypothesis the elucidation of this subject must await considerable further factual contribution.

#### SUMMARY

Infarction of the kidney of the rat is followed by hypertension and by necrotizing and inflammatory arterial lesions.

Partial infarction of one kidney was followed more promptly by hypertension than was total infarction of one kidney. Total infarction of one kidney, in fact, did not produce hypertension in 50 per cent of the animals tested. The foregoing observations may be explained by the difference in the anatomic relations of the anastomosing blood vessels in the 2 cases.

The conclusion is drawn that the necrotic renal tissue is the source of vasopressor and necrotizing agents which are absorbed into the blood stream.

The hypothesis is offered that the vessels become so sensitized to these substances that the vascular reactions, both structural and functional, to these or other, similar substances entering the blood in minimal amounts as a result of normal metabolism become exaggerated and prolonged.

The amount of active renal tissue remaining has an influence in modifying the effects of infarction.

## AN ANALYSIS OF THE KLIPPEL-FEIL SYNDROME

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IN 1912 Klippel and Feil described the pathologic anatomy of absence of the neck in a 46 year old man.<sup>1</sup> The anatomic basis of the syndrome since known by their names consists essentially in a congenital fusion and numerical reduction of the cervical vertebrae. Since the original description of this rare condition, most of the communications have been reports of clinical cases of a less extreme type. The three characteristic clinical features of the syndrome are shortness of the neck, limitation of movement of the head and lowering of the hair line.

In the present paper I shall discuss a theoretic interpretation of the anatomic basis of the syndrome and its relation to Sprengel's deformity and to certain neurologic disorders and consider a possible mechanism whereby such compound defects may take place. A new clinical case is recorded.

### REPORT OF A CASE

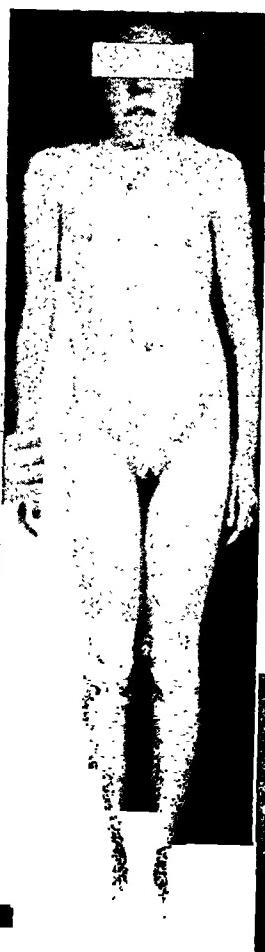
The patient is a girl of 10 years; her neck appears short and broad, and the free edge of the trapezius muscle passes directly from the occiput to the lateral end of the clavicle and gives the neck a webbed appearance (figs. 1 and 2). There is marked limitation of the movement of the head in all directions particularly of lateral bending. The hair line is lower than normal. With the exception of a deformity of the second upper right molar tooth, the teeth are normal. The uvula is bifid. A minor degree of bimanual synkinesia and a slight defect of hearing of the right ear are present.

The roentgenograms reveal in the whole cervical portion of the spinal column a malformation which consists of a synostosis from the second cervical to the first thoracic vertebra and is accompanied by marked shortening (figs. 3 and 4). The posterior arch of the atlas is fused to the laminae and the spine of the axis, and the succeeding four cervical spines are also fused together. Several of the transverse processes are similarly affected. It is possible that there is a cleft in the neural arches of the first two vertebrae. The articulation between the atlas and the occipital condyles is free. There are apparently six vertebral segments which appear compressed and widened, especially in the upper half of the column. It is difficult to identify the individual bodies of the vertebrae, and the number of segments is suggested by the number of intervertebral foramina, which are clearly shown. Bilateral cervical rib is present.

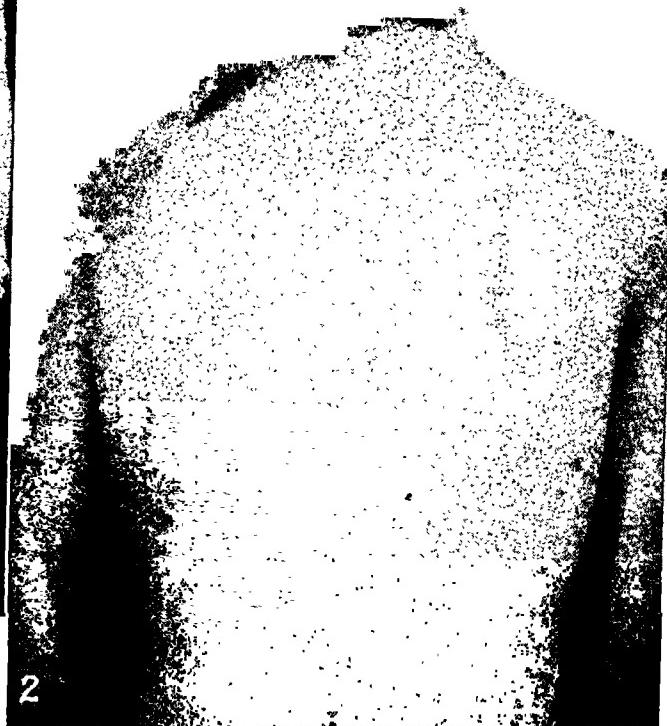
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From the Department of Anatomy, King's College.

1. Klippel and Feil, A.: Bull. et mém. Soc. anat. de Paris 87:185, 1912; Nouv. iconog. de la Salpêtrière 25:223, 1912; Bull. et mém. Soc. d'anthrop. de Paris 3:101, 1912.



2



Figs. 1 and 2.—The width and the shortness of the neck, are shown, in contrast to the slender build of the patient.



3



Figs. 3 and 4.—The roentgenograms illustrate the shortening, the synostosis and the irregularity of the cervical region. Indications of the individual segments are apparent, as shown by the presence of the intervertebral foramens.

## PATHOLOGIC ANATOMY

Postmortem studies of the Klippel-Feil syndrome are rare. There is a small number of descriptions of anatomic specimens in which the osseous deformity only is considered. In the original description of an extreme example the cervical vertebrae were considered to be absent, the fused cervical mass consisting of the upper four thoracic vertebrae. In the 2 cases described by Mitchell,<sup>2</sup> the atlas was fused to the occiput, and the intervertebral disks were irregular or obliterated. In other cases the vertebrae are fused either throughout or in the lower cervical region only, or the fusion may involve the upper three or four thoracic vertebrae. The bodies have been found to be ossifying from two widely separated centers, and the anterior wall of the vertebral canal may be several times its normal width. In the probable cases of Fairbank<sup>3</sup> and those of Laignel-Lavastine and Miget<sup>4</sup> the number of cervical vertebrae seemed to be four. The same number of very deformed and noticeably widened vertebrae appeared in the roentgenogram in a case observed by Crouzon and Liège.<sup>5</sup> Feller and Sternberg<sup>6</sup> expressed the belief that the number of segments can be determined by counting the transverse processes and remarked that a numerical reduction cannot be confirmed.

Spina bifida is frequently found. According to Mitchell,<sup>2</sup> it is of a false type, as some of the laminas curve down to meet in the midline in a fused mass in the lower cervical region, so that a triangular opening is formed, with the apex directed downward and the base toward the occiput. The triangular deficiency in the arches is covered by a layer of fibrous tissue, and occasionally it extends to the upper thoracic region. The occipital bone may be closely approximated to the defect (Feller and Sternberg<sup>6</sup>) and this approximation appears to be related to the cervical lordosis. As a rule, there is no meningocele. An exception is seen in the case of Latto,<sup>7</sup> who commented on the presence of a meningocele in the region of the occiput. Feller and Sternberg<sup>6</sup> observed in 1 of their cases that the hindbrain region was displaced into the upper part of the vertebral canal and noted that this displacement was similar to that found in the Arnold-Chiari malformation.

2. Mitchell, H. S.: Arch. Dis. Childhood **9**:213, 1934.

3. Fairbank, H. A. T.: Brit. J. Surg. **4**:553, 1914.

4. Laignel-Lavastine, M. M., and Miget, A.: Rev. neurol. **1**:782, 1930.

5. Crouzon, O., and Liège, R.: Bull. et mém. Soc. méd. d. hôp. de Paris **52**:917, 1928.

6. Feller, A., and Sternberg, H.: Virchows Arch. f. path. Anat. **285**:112, 1932.

7. Latto, D.: Brit. M. J. **1**:669, 1942.

Jarcho and Levin<sup>8</sup> reported that sections of the spinal cord revealed normal structure. On the other hand, Avery and Rentfro<sup>9</sup> stated that in their case sections of the upper part of the spinal cord showed it to be almost divided into two parts by a deep anterior fissure, that lower sections revealed two anterior fissures and that some sections showed doubling of the central canal and gliosis of a moderate degree.

Fusion and deformities of the ribs occur (Ingelrans and Piquet<sup>10</sup>), and cervical ribs are often present. In cases of the Klippel-Feil syndrome with associated high scapula, the latter is smaller than normal, the infraspinous parts being relatively more affected, and so it resembles the normal fetal scapula in the relation between the vertical and the transverse diameter. A bar of cartilage or bone or a band of fibrous tissue may attach the vertebral border of the scapula to the lamina of a cervical vertebra. Areas of embryonic myoblastic tissue were found throughout the aplastic muscles in a 6 week infant dissected by Middleton.<sup>11</sup>

Other abnormalities occur, some of which clearly depend on the primary cervical lesion, such as torticollis, facial asymmetry, lowered nipple line, kyphosis and thoracic scoliosis, the last occasionally due to a hemivertebra. Also described are abnormal dentition (Willard and Nicholson<sup>12</sup>), cleft palate (Nobel and Frawley<sup>13</sup>), micrognathia (Demeler<sup>14</sup>), hydrocephalus (Feller and Sternberg<sup>6</sup>), strabismus (Thomson<sup>15</sup>), platybasia (Furst and Ostrum,<sup>16</sup> Merio and Risak<sup>17</sup>), pulmonary stenosis (Bizarro<sup>18</sup>), patent foramen ovale and interventricular septum (Mitchell<sup>2</sup>).

#### ETIOLOGY

The consensus regarding the cause of the Klippel-Feil syndrome is that it is a disorder of segmentation. Views of the cause and the pathogenesis vary. They include dysplastic factors resulting in inhibition or necrosis of chondrogenic centers of the vertebrae (Jarcho and Levin<sup>8</sup>), morbid conditions of the parent (Rechtman and Horwitz<sup>19</sup>),

8. Jarcho, S., and Levin, P. M.: Bull. Johns Hopkins Hosp. **62**:216, 1938.
9. Avery, L. W., and Rentfro, C. C.: Arch. Neurol. & Psychiat. **36**:1068, 1936.
10. Ingelrans, P., and Piquet, J.: Rev. d'orthop. **15**:297, 1928.
11. Middleton, D. S.: Edinburgh M. J. **41**:401, 1934.
12. Willard, DeF. P., and Nicholson, J. T.: Ann. Surg. **99**:561, 1934.
13. Nobel, T. P., and Frawley, J. M.: Ann. Surg. **82**:728, 1925.
14. Demeler, W.: Ueber familiäre missbildungender Wirbelsäule, Inaug. Dissert., Münster, 1933.
15. Thomson, J.: Arch. Dis. Childhood **12**:127, 1937.
16. Furst, W., and Ostrum, H. W.: Am. J. Roentgenol. **47**:588, 1942.
17. Merio, P., and Risak, E.: Ztschr. f. klin. Med. **126**: 455, 1934.
18. Bizarro, A. H.: Lancet **2**:828, 1938.
19. Rechtman, A. M., and Horwitz, M. T.: Am. J. Roentgenol. **43**:66, 1940.

sequelae of infections of infancy or of intrauterine life (Rebierre<sup>20</sup>), weakening of the hereditary powers in the parent (Nobel and Frawley<sup>18</sup>) and injurious influences resulting in inhibition of regressive or progressive processes during development (Foggie<sup>21</sup>); in some cases the syndrome is regarded as syphilitic in origin (Roger, Arnaud and Audier<sup>22</sup>). Occasionally the numerical reduction of the vertebrae, spina bifida or a defect in the intervertebral disks is considered to be the primary factor in the malformation. Kallius<sup>23</sup> based a classification of the anomalies of the vertebral axis on the theory of Rosenberg, which relates the inconstant morphologic character of the human vertebral column to its phylogenetic development and probable future forms and suggests that the deformities can be explained by stability or instability of structure. This theory is considered by Kallius to be more satisfactory than a theory based on the assumption of accidental disturbances of the chromosomal determinants.

It has been stated that the Klippel-Feil syndrome is not hereditary or familial (Nobel and Frawley<sup>18</sup>) even by those (Thomson<sup>15</sup>) who have pointed out the cases (cited by Feil<sup>24</sup>) in which the syndrome was observed in father and son and in mother and 3 children (cited by Sicard and Lermoyez<sup>25</sup>); occasionally cited is a case reported by Kallius,<sup>23</sup> in which the syndrome occurred in father and daughter. To these may be added a probable case reported by Sick<sup>26</sup> in which a sister of the patient's father and other relatives on the father's side were affected, the cases of Jarcho and Levin<sup>8</sup> in which a brother and a sister were involved, with a minor vertebral defect in the mother, the cases of Demeler<sup>14</sup> in which at least 2 brothers and 1 sister showed the syndrome, those of Clemmensen<sup>27</sup> in which the patients were mother and daughter, and 1 of Bizzaro's<sup>18</sup> in which the father and the mother were both affected, as well as a probable case reported by Bar,<sup>28</sup> in which brother and sister were the patients. Hangarter and Dieker<sup>29</sup> reported the case of a female with the Klippel-Feil syndrome whose sister, both parents and other relations showed skeletal defects. When the cases in which no apparent hereditary or familial element was shown are considered, the constancy of the deformity

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20. Rebierre, P.: Presse méd. **31**:452, 1923.
  21. Foggie, W. E.: Edinburgh M. J. **42**:421, 1935.
  22. Roger, H.; Arnaud, M., and Audier, M.: Marseille-méd. **1**:233, 1934.
  23. Kallius, H. U.: Arch. f. orthop. u. Unfall-Chir. **29**:440, 1931; **31**:287, 1932.
  24. Feil, A.: *L'absence et la diminution des vertèbres cervicales*, Thesis, Paris, no. 212, 1919.
  25. Sicard, J. A., and Lermoyez, J.: Rev. neurol. **30**:71, 1923.
  26. Sick, P.: Deutsche Ztschr. f. Chir. **67**:556, 1902.
  27. Clemmensen, V.: Acta radiol. **17**:480, 1936.
  28. Bar, P.: Bull. Soc. d'obst. de Paris **6**:425, 1903; **7**:250, 1904.
  29. Hangarter, W., and Dieker, W.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **21**:236, 1937.

observed suggests a genetic basis. It may be added that Neuhof<sup>30</sup> and more recently Schwarzweller<sup>31</sup> gave evidence of a hereditofamilial incidence of the closely related Sprengel deformity.

There is also general agreement that the incidence is equal in males and females (Bauman<sup>32</sup>; Foggie<sup>21</sup>; Willard and Nicholson<sup>12</sup>), though some (Nobel and Frawley<sup>13</sup>) have stated that it is more common in males. However, if only those cases which appear to be genuine examples of the Klippel-Feil syndrome and which present closely similar anatomic features are selected, it appears that there is a preponderance of females; though the total number of cases is small, this preponderance is probably significant. In such a selection of 52 cases in the literature there are 34 females and 18 males. This higher incidence in females is supported by the observation of Gilmour.<sup>33</sup>

Some authors require a numerical reduction of the vertebral axis to complete the syndrome, but many cases are classified as instances of Klippel-Feil syndrome without numerical reduction and in others its presence is difficult to prove (Mitchell<sup>2</sup>). Reduction occurred in only 1 of the 6 cases of Feller and Sternberg.<sup>6</sup> They regarded the reduction in this case as physiologic. But it seems that reduction is present more frequently than can be accounted for on physiologic grounds. In a study of 1,420 vertebral columns LeDouble<sup>34</sup> observed only 2 in which the number of cervical vertebrae was reduced to six. Avery and Rentfro<sup>9</sup> expressed the view that numerical reduction is an essential part of the syndrome, and at the same time they agreed with Müller<sup>35</sup> that it occurs when the growth of the normally developing parts forces the misplaced and abnormal parts into a mass formation as the body grows older. Reduction of the segments in the cases classified as Sprengel's deformity has been reported by Critchley,<sup>36</sup> Niederle,<sup>37</sup> Fairbank<sup>3</sup> and others. Many cases which some authors would consider to be instances of the Klippel-Feil syndrome are classified by others as cases of Sprengel's deformity. Further examples are described under still other headings (Sever<sup>38</sup>). Heidecker<sup>39</sup> pointed out the difficulty of distinguishing the two conditions when Sprengel's deformity is bilateral. A well known

30. Neuhof, H.: Am. J. Dis. Child. **7**:357, 1914.

31. Schwarzweller, F.: Ztschr. f. menschl. Vererb.- u. Konstitutionslehre **20**: 350, 1937.

32. Bauman, G. I.: J. A. M. A. **98**:129, 1932.

33. Gilmour, J. R.: J. Path. & Bact. **53**:117, 1941.

34. LeDouble, A.-F.: Gaz. méd. du centre **17**:29, 1912.

35. Müller, W.: Deutsche Ztschr. f. Chir. **242**:94, 1933.

36. Critchley, M.: Brit. J. Surg. **14**:243, 1926.

37. Niederle, B.: Časop. lék. česk. **63**:1743, 1924; cited by Du Toit.<sup>41</sup>

38. Sever, J. W.: Boston M. & S. J. **186**:799, 1922.

39. Heidecker, H.: Beitr. z. klin. Chir. **144**:303, 1928.

early clinical case of the Klippel-Feil syndrome is that of Clarke,<sup>40</sup> and the probable cases of Bar<sup>28</sup> and Sick<sup>26</sup> have been mentioned. A cervical osseous deformity which now would probably be classified by many as the Klippel-Feil syndrome was described by Rokitansky in 1850. Du Toit<sup>41</sup> remarked that cases of high scapulas were reported by Underburg in 1693, and doubtless other early cases could be found which modern authors would place in one of the two categories. Böhm<sup>42</sup> expressed the opinion that the Klippel-Feil syndrome is not an entity and claimed priority for a description of the cervical malformation published in 1909. These factors make it difficult to assess the exact number of cases described, the total ranging from 50 to a recent estimate of 133 made by Rechtman and Horwitz<sup>19</sup>. A large proportion especially of the earlier cases are described in the French literature (Feil<sup>24</sup>; Dubreuil-Chambardel<sup>43</sup>; Lance<sup>44</sup>; Michel and Nicolleau<sup>45</sup>; Crouzon and Martin<sup>46</sup> and others).

Though Feller and Sternberg<sup>6</sup> regarded nervous defects as rare, it will be observed that in addition to the pathologic anatomy already described, other disorders found are mental weakness and deaf-mutism (Foggie<sup>21</sup>; Bauman<sup>32</sup>), nystagmus (Latto<sup>7</sup>), late spastic paralysis (Guy-Laroche and Klotz<sup>47</sup>; Baruch<sup>48</sup>), late syringobulbia and syringomyelia (Kallius<sup>23</sup>; Critchley<sup>36</sup>; Du Toit<sup>41</sup>), central facial weakness (Furst and Ostrum<sup>16</sup>), bimanual synkinesia (Bauman<sup>32</sup>; Willard and Nicholson<sup>12</sup>) and late involvement of the sympathetic nervous system (Turner, Shoulders and Scott<sup>49</sup>). Rebierre<sup>20</sup> described a case in which he found partial anesthesia of the soft palate, right lingual hemiatrophy, a zone of cutaneous anesthesia at the base of the neck and paralysis of the right vocal cord. Owing to the rarity of bimanual synkinesia alone and the rather frequent association with the Klippel-Feil syndrome, it is stated (Bauman<sup>32</sup>) that synkinesia should be regarded as part of the syndrome, but Willard and Nicholson<sup>12</sup> considered that the two are independent.

It is clear from a review of the cases in the literature that their most constant features are synostosis, flattening of the vertebrae; narrowing or obliteration of the intervertebral disks, shortening of the cervical spine

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40. Clarke, J. J.: *Lancet* **2**:1350, 1906.
  41. Du Toit, F.: *Brain* **54**:421, 1931.
  42. Böhm, M.: *Zentralbl. f. Chir.* **58**:2019, 1931.
  43. Dubreuil-Chambardel, L.: *Presse méd.* **29**:353, 1921.
  44. Lance, M.: *Presse méd.* **29**:27, 1921.
  45. Michel, A., and Nicolleau: *Marseille-méd.* **59**:394, 1922.
  46. Crouzon, O., and Martin, R.: *Rev. neurol.* **1**:270, 1923.
  47. Guy-Laroche and Klotz, B.: *Rev. neurol.* **2**:47, 1933.
  48. Baruch, R.: *Ztschr. f. d. ges. Neurol. u. Psychiat.* **139**:462, 1932.
  49. Turner, E. L.; Shoulders, H. S., and Scott, L. D.: *Am. J. Roentgenol.* **40**:43, 1938.

and spina bifida. In at least some cases it appears that the characteristically lowered hair line is due to the same type of hirsutism as that found in other regions in spina bifida occulta, as has been noted by Rebierre.<sup>20</sup> Reduction occurs but it is probably less common than the roentgenograms suggest, and it is doubtful if it is an essential part of the disorder. It is also clear that the variations seen in the syndrome are chiefly those of degree.

#### COMMENT

The principle of gastrulation, according to which the germ layers are formed, is common to all vertebrates, though the mechanisms may differ. These mechanisms, dynamic processes involving coordinated cellular movements, have been studied in those forms in which gastrulation takes place in a manner similar to that of gastrulation in man. The position of the materials which normally will become the somites, myotomes and other structures has been mapped in considerable detail (Pasteels<sup>50</sup> and others), following the application by Vogt of the method of vital staining, by colored marks. The position of the areas varies somewhat in different creatures which possess a disklike form in early stages, as man, though in gastrulation the essential feature is the relative movements which take place rather than the form of the map.

Let it be assumed that a disturbing factor is present over the area where the rostral end of the primitive streak appears. Whether or not the streak represents a part of gastrulation, such a factor would involve approximately the prechordal plate, the rostral end of the notochord, the cervical somitic mesoblast and the mesoblast of the head and neck region from which the myotonic muscles of the neck and other structures are developed. That is, it would affect the earliest structures to be involved in gastrulation. Such a factor may arise on fertilization and could become established even before the first division of the egg. Environmental factors acting very soon after fertilization and before cleavage have been shown to produce localized defects in *Heteroclitus* (Hinrichs and Genther<sup>51</sup>); these defects were produced by ultraviolet rays acting for a short period and the results varied in type; the effect on axial structures in such cases is generally manifested by a tendency toward duplication.

In this connection it may be added that duplication of the cervical spine in the Klippe-Feil deformity cannot be excluded; the roentgenograms in certain cases resemble those of a partial duplication. Feller and Sternberg<sup>6</sup> described a fetus in which sections of the malformed part of the vertebral column showed that the remainder of the notochord was divided into two parts. In experimental animals, doubling of this

50. Pasteels, J.: Arch. Biol. 47:205, 1936.

51. Hinrichs, M. A., and Genther, I. T.: Physiol. Zool. 4:461, 1931.

structure is followed by vertebral duplication. Some cases of the Klippel-Feil syndrome may represent a primary duplication of the axis with subsequent incomplete reunion. Early experimental findings have shown that if the mesodermal axis is duplicated, the neural organ is subject to the same change by a process of induction. Thus in this connection the finding of Avery and Rentfro<sup>9</sup> acquires special significance; in their case the spinal cord presented a doubling of the central canal and other features suggesting duplication.

Gabriel<sup>52</sup> has shown experimentally that environmental conditions (in this case variations in temperature) resulted in numerical variations in the somites. A lowered temperature produced an actual increase in the number of somites; a raised temperature decreased the number. The reduction tends to occur at the caudal end of the axis and is related to the amount of material available for somite formation and to the speed of the developmental reactions. Differentiation and growth can be independent of each other. If the former becomes precocious relative to the somite separation and growth, fusion of segments as well as numerical reduction may occur. It is pointed out that as mesodermal cells become differentiated they may lose their ability to become separated into somites. Thus an acceleration of differentiation can result in fusion of segments. A generalized influence as far as the segments are concerned produces an effect similar to that which occurs in the Klippel-Feil syndrome, namely, fusion and reduction in their number. It might be suggested that an increase in the temperature of the environment of the embryo would have the same effect. An increase in temperature is usually followed by raised metabolic activity. If this is the basis of the anomalies of segmentation, a genetic factor which raises metabolic activity in a similar manner could produce a similar result. It appears that, though the reduction and the fusion are due to distinct immediate causes, they are associated under a generalized influence. Feller and Sternberg<sup>6</sup> have suggested that it is not a true fusion but a disorder associated with a defect in the disks. Partsch<sup>53</sup> expressed the belief that the disorder arises when the vertebral column is represented by a cartilaginous bar. The latter condition, however, is not present at any time during development. Though too close an analogy between man and *Heteroclitus* should not be drawn, the principle may well be similar; the experimental production in the Axolotl of complex malformations involving deficiencies and reductions similar to those of genetic origin in man is described by Brandt.<sup>54</sup> But the Klippel-Feil syndrome often involves more than fusion and a numerical reduction of segments. An

52. Gabriel, M. L.: *J. Exper. Zool.* **95**:105, 1944.

53. Partsch, F.: *Arch. f. orthop.- u. Unfall-Chir.* **24**:199, 1927.

54. Brandt, W.: *J. Exper. Biol.* **20**:117, 1944.

explanation is required for the selective locality of the abnormality at the cranial end of the axis as well as for the frequently associated high scapula and muscle defects. It appears that the disturbance occurs at a specific time or over a specific area. In this case it is possible that fusion may be independent of the numerical reduction. Thus it seems that such a mechanism as an alteration of the temperature of the environment or a general effect exercised on metabolism by a genetic factor cannot account for all cases of the syndrome. The extent and the position of the disturbance in the Klippel-Feil deformity may be explained if one assumes a factor whose action is local, even if the action is a nonspecific one, such as an acceleration in the developmental rate. A factor acting in time, that is during the gastrulation process for the cervical structures, could produce a similar result. In either case there is a certain specificity in space or time; this specificity implies a genetic factor. Points in favor of the temporal conception will be discussed below and are obtained from the consideration of the relationship between the Klippel-Feil syndrome and Sprengel's deformity.

The appearance of the effects of the early disturbance may be placed shortly after gastrulation, that is with the appearance of the somites rather than with that of the definitive vertebrae. This is suggested by those cases in which the vertebral bodies are wide and possess bilateral ossification centers such as might result from arrest in development with ossification in the bilateral chondrification centers. At the same time it would appear that the spina bifida is at least partly mechanical. If the arches, which ossify some time before the bodies, were to differentiate in contact with each other because of the lack of depth in the bodies, fusion might be assumed. Subsequent differential growth in the bodies could account for the type of spina bifida which is described.

There is clearly a relation between congenital high scapula, or so-called Sprengel's deformity, and the Klippel-Feil syndrome, as the former usually appears in association with a cervical malformation similar to that found in the latter. The converse is however not always true. Lichtenstein<sup>55</sup> in a discussion of observations reported by Gustafson and Oldberg suggested that platybasia is a connecting link between the two anomalies. Some authors (Bizarro<sup>18</sup>) suggested that the Klippel-Feil syndrome is an advanced stage of Sprengel's deformity, but this appears to imply not only that there is a direct relationship of a progressive nature between the two conditions but that the Klippel-Feil syndrome is always accompanied by Sprengel's deformity. This does not appear to be so, and, further, the degree of severity of the one is not necessarily accompanied by a corresponding degree of severity of the other. The

55. Lichtenstein, in discussion on Gustafson, W. A., and Oldberg, E.: Arch. Neurol. & Psychiat. 54:1184, 1940; cited by Furst and Ostrum.

relation seems rather to be one of common origin. The tissues of the scapular region and those of the cervical vertebrae are developed to some extent from a common mesodermal substratum, although they are not differentiated simultaneously. A disturbance producing the Klippel-Feil syndrome may have ceased to act by the time the scapular structures appear, with the result that a pure Klippel-Feil syndrome is produced. A similar type of disturbance occurring later might affect the developing scapular structures and produce Sprengel's deformity. If at the same time it affects the axis, the latter would have passed the stage at which the typical Klippel-Feil syndrome could be produced. This hypothesis accounts for the peculiar relationship between the two conditions. A similar connection may exist between the Klippel-Feil deformity and iniencephaly. Gilmour<sup>53</sup> stated that the latter is a more severe form of the deformity seen in the Klippel-Feil syndrome. In support of this belief that the two conditions are the same, Gilmour cited as examples of iniencephaly 3 cases classified by Feller and Sternberg<sup>6</sup> as examples of the Klippel-Feil syndrome. There is to be found in both infants and adults a number of severe forms of the Klippel-Feil syndrome which do not possess the characters of iniencephaly; notable among them is the original form described by Klippel and Feil.<sup>1</sup> It is agreed that certain cases described by Feller and Sternberg may be instances of iniencephaly and that the latter may have the characters of the Klippel-Feil deformity in the cervical region but that the converse does not apply.

With regard to the teeth and the mouth Schröder<sup>56</sup> remarked on the association of cleft palate and malformations of the spinal column which is observed in certain families. It was noted in the case reported earlier in this article that there was a minor defect related to cleft palate, that is, a bifid uvula. Platt<sup>57</sup> showed that the tissue underlying the oral region is partly composed of material of neural crest origin, and the suggestion is made that it may be the source of the primitive dentine. This mesectoderm material migrates from the dorsal region of the neural tube to the buccal region. A disturbance of the mesoderm of the head region might have the effect of arresting some part of this material. Thus the cleft palate may well be the result of the branchial arch structures of the palate growing into oral ground tissue which is defective. Failure of eruption or malformations of the teeth may also be correlated indirectly with the same early mesodermal disorder.

. There does not seem to be a description of the pathologic anatomy of the ears in those cases of the Klippel-Feil syndrome with deaf-mutism, though a deformity of the ear in the form of absence of the external meatus is described by Ingelrans and Piquet.<sup>10</sup> When congenital deaf-

56. Schröder, C. H.: Beitr. z. klin. Chir. **169**:402, 1939.

57. Platt, J. B.: Morphol. Jahrb. **25**:377, 1897.

mutism is the chief defect, its pathologic character varies. Schiebe's type comprises more than half the cases. In connection with such auditory defects Bartelemez and Evans<sup>58</sup> described a mesectoderm proliferation from the hindbrain region in man, which probably contributes to the visceral arch mesenchyme. Further, Stone<sup>59</sup> showed experimentally that removal of neural crest material will result in defects of the visceral arch skeleton. Since the auditory ossicles are formed from visceral arch material, it is possible that the factor discussed in connection with the adynamic failure in somitic morphogenesis might be responsible for the retardation of migration of mesectoderm which results in defective differentiation in the middle ear. Such a disturbance in development, if severe, would produce a defect not dissimilar to Siebermann's type of congenital deafness. If the deaf-mutism is sensory and at the same time not coincidental, an explanation might be provided by a consideration of the dependent relation between the otic vesicle and the head mesoderm; that is, the latter takes part in determining the appearance of the former. The presence of the head mesoderm is also responsible for the continued normal development of the otic vesicle (Kaan<sup>60</sup>). Since it has been shown by Mangold<sup>61</sup> that in some vertebrates the removal of certain parts of the mesoderm underlying the cranial end of the neural plate results in cyclopia, the formation of the eyes is also related to the underlying mesoderm; however, in man sensory defects of a character comparable to deaf-mutism, that is, optic aplasia, have not been described in these cases. There is a distinction, however, apart from the species difference, in that the underlying material involved in the induction of the ear may be related to the proximity of those precervical somites which are taken up in the basis cranii. It is admitted that such auditory, dental and palatal defects as those described in connection with the Klippel-Feil syndrome may be coincidental, but the foregoing facts appear to be suggestive.

The association of synkinesia with the syndrome has been mentioned. Baigley<sup>62</sup> remarked that all patients with hemiplegia show it and cited Stewart for the information that it is common to see conjugate movements in spastic hemiplegia, and that they are really tonic reflex reactions similar to those in decerebrate animals; the movements are reflex, due to changes in muscle tone. The type of synkinesia described in the Klippel-Feil syndrome does not appear to be of this type. Willard and Nicholson<sup>12</sup> and others have remarked that every movement of one hand

58. Bartelemez, G. W., and Evans, H. M.: Contrib. Embryol. **17**:1, 1926.

59. Stone, L. S.: J. Exper. Zool. **44**:95, 1926.

60. Kaan, H. W.: J. Exper. Zool. **78**:59, 1938.

61. Mangold, O.: Ergebn. d. Biol. **7**:193, 1931.

62. Baigley, in discussion on Bauman.<sup>22</sup>

is copied almost exactly by the other. Climbing a ladder can be impossible because of the synchronized movements of the hands. Thus it would appear that there is a considerable element of physiologic movement involved. If so, it may be explained on the basis of certain experimental results. The induction and formation of the central nervous system in many vertebrates have been shown to have some dependent relation with the underlying mesoderm during early development. Among these vertebrates mammals may be included, as Töro<sup>63</sup> has shown that neural induction can also occur in this species. By a more refined analysis in suitable cases Holtfreter<sup>64</sup> revealed that there is also a dependent relation of the internal configuration of the spinal cord with the disposition of the immediately surrounding tissues. If in mammals, as well as induction, there is also a similar relation with the disposition of surrounding tissues, the synkinesia may in this manner be related to the primary disorders responsible for the defects in the mesodermal structures. Such disorders of internal configuration as incomplete bilateral segregation of the cells of origin of the brachial plexus might be considered.

#### CONCLUSIONS

From the example of the Klippel-Feil syndrome presented and from the case reported in the literature it is concluded that the essential features of the cervical deformity are synostosis of two or more cervical vertebrae and flattening and widening of the vertebral bodies. A numerical reduction of the vertebrae is an incidental rather than an essential part of the disorder, as is spina bifida. The latter depends largely on the degree of abnormality of the vertebral bodies. There is evidence that the anomaly has a genetic basis. A number of pathologic conditions which have been found in association with the osseous deformity of the syndrome receive an explanation in the light of recent observations in the field of experimental embryology.

63. Töro, E.: *J. Exper. Zoöl.* **79**:213, 1938.

64. Holtfreter, J.: *Arch. f. exper. Zellforsch.* **15**:281, 1934.

## OVARIAN INVOLVEMENT IN HODGKIN'S DISEASE

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THE infrequency of ovarian involvement in the course of Hodgkin's disease is apparent from a brief perusal of the voluminous literature of this disease. No mention of such occurrence was noted in any of several standard textbooks of general, surgical and gynecologic pathology. In numerous reports of unusual ovarian tumors no instance of Hodgkin's disease was encountered. A closely related lesion was reported in the first 50 cases filed with the Ovarian Tumor Registry of the American Gynecological Society.<sup>1</sup> A 16 year old patient (case 28) had lymphosarcoma of the ovary secondary to intestinal lymphosarcoma. The accompanying photomicrograph reveals an infiltration strikingly similar in its general characteristics to that of our cases. Reviews dealing with Hodgkin's disease and reports representing relatively large series of cases, as a rule, do not refer to the ovary. In Wallhauser's<sup>2</sup> review ovarian involvement is mentioned without specific reference to the authors cited (Wallhard and Mousson). Similarly, Fabian<sup>3</sup> credited Herard with a report of ovarian involvement but failed to include this author in his bibliography. Over a thousand references are represented in the two preceding reviews. Jackson and Parker,<sup>4</sup> reporting the autopsy observations of 112 cases of Hodgkin's disease, mentioned involvement of the ovary in 2 cases. In one case the involvement was microscopic in character; in the other (tabulated) the extent of involvement was not indicated. Gemmell<sup>5</sup> in a report based on a study of 57 cases of Hodgkin's disease of the female theorized that ovarian hypofunction may be responsible for the occurrence of the disease in women; he described no lesions of the ovary to substantiate this concept, which was based chiefly on the history of reduced or suppressed menstruation in several cases.

That the infiltration of the ovary in Hodgkin's disease can be of considerable extent is illustrated in the cases reported in the following

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From the Department of Pathology, University of Pittsburgh, and Presbyterian and Woman's Hospitals.

1. Novak, E.: Am. J. Obst. & Gynec. **48**:861, 1944.
2. Wallhauser, A.: Arch. Path. **16**:522, 1933.
3. Fabian, E.: Centralbl. f. allg. Path. u. path. Anat. **22**:145, 1911.
4. Jackson, H., Jr., and Parker, F., Jr.: New England J. Med. **231**:35, 1944.
5. Gemmell, A. A.: J. Obst. & Gynaec. Brit. Emp. **30**:373, 1923.

pages. In each case the lesion of the ovary was misinterpreted as a primary tumor from its gross appearance. In case 1, the resident physician who did the autopsy submitted a gross anatomic diagnosis of "carcinoma, probably primary in the ovary, with widespread secondary involvement of the abdominal lymph nodes." Of particular interest is case 2, in which the surgeon, an experienced gynecologist, noted during abdominal exploration an ovarian tumor of such size that it was considered primary in that organ, while the neoplastic enlargement of the mesenteric lymph nodes was considered to be secondary to the ovarian lesion.

#### REPORT OF CASES

CASE 1.—Mrs. S. G., a 69 year old white woman, was admitted to the Presbyterian Hospital, service of Dr. A. H. Colwell, Dec. 20, 1944. She complained of abdominal pain and a cough of three weeks' duration. In the previous six months she had lost 30 pounds (13.5 Kg.) in weight.

Examination revealed a poorly nourished patient with evidence of vitamin deficiency. None of the superficial lymph nodes were enlarged. The chest was essentially normal. The abdomen was distended and showed evidence of free fluid. An indefinite mass was noted in the epigastrium. The temperature on admission was 102 F., and during the following six days elevation was intermittent, sometimes reaching 103 F. Signs of pulmonary consolidation developed, and after one week of treatment with sulfamerazine her temperature returned to normal. She was unable to take food, became progressively weaker and died Jan. 9, 1945.

Laboratory studies gave results that were essentially normal except for moderate anemia. The blood counts on several occasions revealed the number of red blood cells to vary between 3,070,000 and 3,650,000 per cubic millimeter; the hemoglobin content ranged from 63 to 73 per cent; the white blood cells, from 5,200 to 12,900 per cubic millimeter. Differential examination of the blood films revealed mature polymorphonuclear leukocytes to constitute from 89 to 94 per cent, lymphocytes from 6 to 10 per cent and monocytes from 0 to 1 per cent.

*Autopsy* (six hours after death).—The body showed evidence of extreme malnutrition. There was no enlargement of any of the superficial lymph nodes. The breasts were atrophic, and the bony thorax was poorly developed. The abdomen was slightly distended, and a poorly defined mass was palpable in the epigastrium. Removal and section of the thoracic organs showed no significant gross abnormalities except for moderately intense pulmonary congestion and edema and small, patchy bronchopneumonic areas in both lungs.

There was 400 cc. of clear ascitic fluid in the peritoneal cavity. Throughout the omentum and the mesentery were numerous prominent lymph nodes, varying from 2 to 5 cm. in diameter. Their cut surfaces were pale, translucent, glistening, fleshy in appearance and studded with small irregular yellow areas of necrosis. The consistency was soft. A large, irregular, coarsely nodular retroperitoneal mass extended from the inferior surface of the diaphragm to the aortic bifurcation. On section numerous lymph nodes were noted, which were firmly adherent to one another and which formed the entire mass. The individual nodes were readily identified and measured from 1 cm. to several centimeters in diameter. Their cut surfaces were similar to those of the mesenteric group. The capsules of all nodes appeared intact, and diffuse interstitial infiltration was not evident. The

pericholecystic and peripancreatic nodes were similarly but less extensively altered. With the exception of the left ovary the abdominal viscera were not noteworthy. There was moderate dilatation of the renal pelvis and of the upper portions of the ureters, both of which were incorporated in the retroperitoneal mass.

The uterus, both fallopian tubes and the right ovary showed advanced involutionary atrophy, the ovary measuring 1.5 cm. in its greatest diameter. It was approximately the size and the shape of a shelled peanut. The left ovary was enlarged in an irregular fashion and measured 4.5 cm. in its greatest diameter. It was located in a normal position and was freely movable, and no enlarged lymph nodes were in close proximity. Its surface was smooth, the capsule tense and its contour irregularly oval. The consistency was moderately soft, and on section a well circumscribed irregular nodular mass measuring 4 by 2 by 2 cm. was noted, replacing the bulk of the stroma, remnants of which were compressed laterally. The cut surface of the tissue was pale and fleshy in appearance, similar to that of the abdominal lymph nodes (fig. 1 A).

*Microscopic Examination.*—Sections of the retroperitoneal mass and of the mesenteric, omental, peripancreatic and pericholecystic lymph nodes revealed a similar picture throughout. A highly cellular process had destroyed the nodal structure, only isolated remnants of which remained. The cells were closely spaced and exhibited no structural pattern. In areas, mature lymphocytes were generously intermingled with the tumor. The tumor cells showed considerable pleomorphism. Most of the cells were large and showed much variation in shape. The cytoplasm was abundant, homogeneous and free of granules (fig. 1 B). For the most part, it stained intensely acidophilic, but amphoteric and lightly basophilic forms were noted. The nuclei for the most part were large, vesicular and often multilobulated. Prominent nucleoli were frequent. Multinucleated giant cells of the Sternberg-Reed type were commonly encountered.<sup>5a</sup> Mitotic figures were numerous. Smaller forms with single delicate vesicular or pyknotic nuclei and pale-staining cytoplasm were common. Many of the tumor cells were within the sinusoidal spaces, but interstitial infiltration was a prominent feature of the reaction. There were large areas of ischemic necrosis; elsewhere, widespread interstitial fibrosis was present.

Sections of ovary revealed two types of lesions. One was similar to that of the abdominal lymph nodes, consisting of large cells of reticuloendothelial type showing scattered mitotic figures and considerable pleomorphism, in which giant cells of the Sternberg-Reed type were prominent. Clusters of these cells were occasionally noted within the lumens of veins.

In other areas the parenchyma was extensively infiltrated and largely replaced by a heavy lymphoid infiltration, associated at times with moderate interstitial fibrosis. The cells were uniform in size, shape and appearance and were typical mature lymphocytes with scant cytoplasm (fig. 1 C). In these areas reticuloendothelial cells were rarely evident. Intermingling of the sarcomatous and lymphoid patterns was noted at the junction of these patterns (fig. 1 D). In areas, stroma of ovarian type containing corpora albicantia served to identify the tissue as ovary.

Lymphoid infiltrations similar to that seen in the ovary were noted in sections of lung, pancreas, epicardium, fallopian tube, serosa of small intestine, kidneys and bone marrow. The infiltration of these organs was purely lymphocytic and of only microscopic proportions, rarely replacing parenchymatous tissue.

5a. The inaccuracy of the eponym "Sternberg-Reed" has been recently pointed out by Symmers (J.A.M.A. 128:1248 [Aug. 25] 1945).

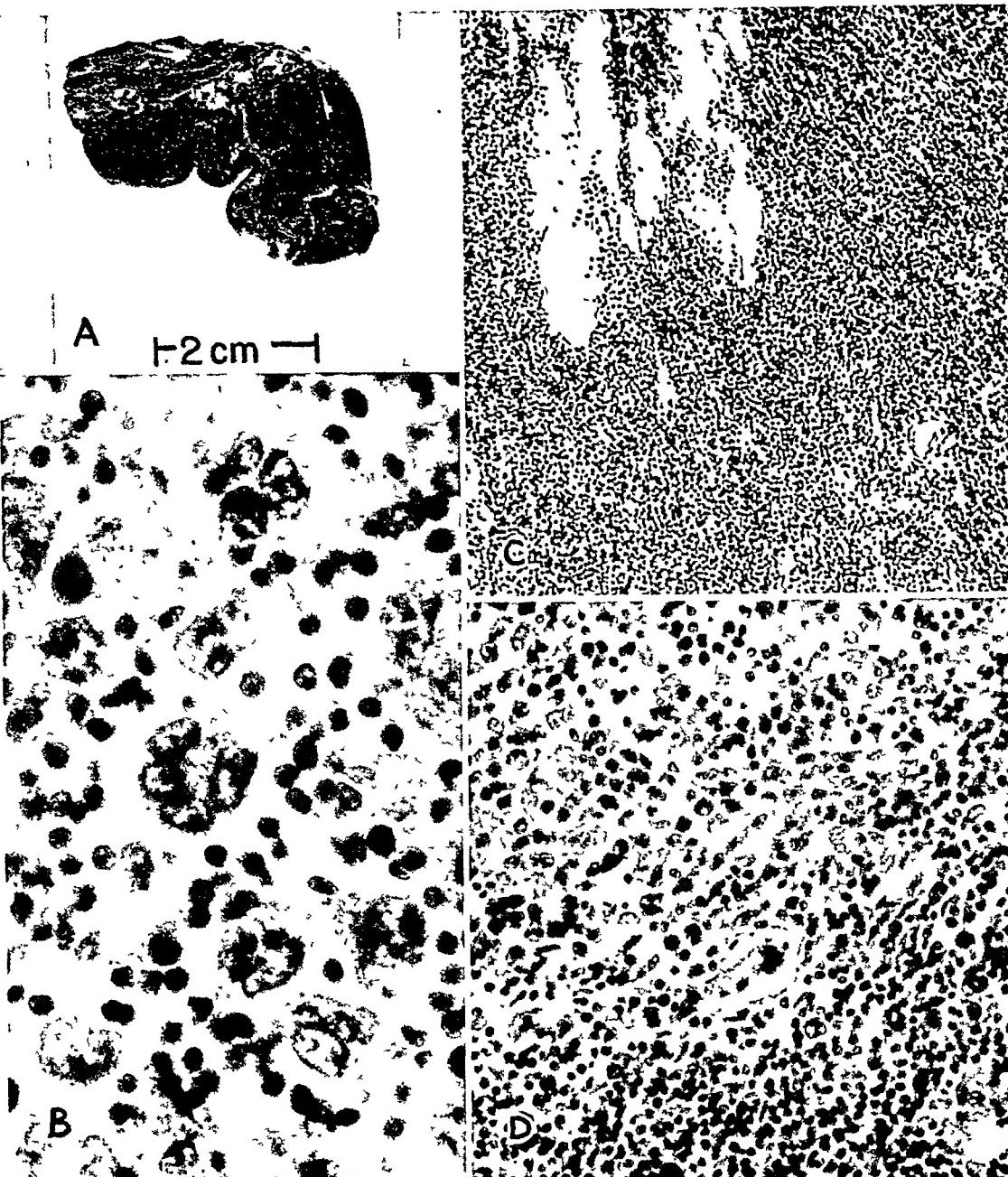


Fig. 1 (case 1).—*A*, ovarian tumor. The cut surface is pale, solid and coarsely lobulated. Displaced ovarian parenchyma appears above.

*B*, lymph node. The large frequently multinucleated sarcomatous cells are infiltrating the lymphoid parenchyma.  $\times 800$ .

*C*, ovarian tumor. There is diffuse lymphoid infiltration around remnants of a corpus fibrosum.  $\times 130$ .

*D*, ovarian tumor. The area represents the transition zone between the sarcomatous and the lymphoid infiltration, the latter appearing below.  $\times 290$ .

CASE 2.—Mrs. V. V., a 35 year old white woman, reported to the Pittsburgh Skin and Cancer Foundation, May 17, 1945, complaining of continuous pressure and cramplike pains in the right lower quadrant of the abdomen, and progressive enlargement of the abdomen of six weeks' duration. On abdominal examination a firm, nontender, orange size mass, dull to percussion, was found in the epigastrium. Pelvic examination revealed an irregular firm nodular mass located in the region of the right ovary. Similar nodules were felt in the cul-de-sac.

Laboratory studies were noncontributory. The red blood cells numbered between 3,520,000 and 3,780,000 per cubic millimeter; the hemoglobin content ranged from 70 to 76 per cent, and the white blood cells numbered from 7,250 to 9,600 per cubic millimeter.

The clinical impression was that of ovarian cancer with metastatic involvement of the abdominal lymph nodes.

The patient was admitted to Woman's Hospital, service of Dr. Alfred A. Pachel, for laparotomy, which was done on June 4. The right ovary and a biopsy specimen of the omentum were removed. A large, solid, orange size mesenteric mass, firm in consistency and fixed on all sides, was noted in the epigastrium. The post-operative surgical diagnosis was primary solid carcinoma of the ovary with secondary involvement of the abdominal lymph nodes. The patient was discharged on the sixteenth postoperative day, following an uneventful postoperative course.

*Pathologic Examination.*—The specimen consisted of an ovary and an irregular piece of omentum.

The ovary was greatly enlarged, measuring 8 by 5 by 4 cm. It was ovoid and covered by an intact thin capsule which was smooth, glistening, opaque, and discolored in areas by irregular subcapsular hemorrhages. The surface was slightly nodular. On section a pale, yellowish white, moderately firm, fleshy-appearing, solid tissue was noted, of uniform character throughout (fig. 2 A).

Within the omental fat were small irregular nodular areas of tissue similar in appearance to that of the ovary.

Sections revealed a thin fibrous capsule, beneath which atrophic remnants of ovarian stroma were noted, containing distinct but often distorted graafian follicles. The parenchymatous and the stromal elements had been largely replaced by a heavy cellular infiltration associated with considerable fibrosis. The infiltration was diffuse, intense and without structural pattern. The fibrosis was of irregular distribution, in some areas extensive and diffuse and productive of heavy depositis of collagen. Elsewhere it was less extensive and disposed as elongated trabeculae which traversed the cellular areas (fig. 2 B).

The predominating cells were of lymphoid type, the great majority being typical mature lymphocytes tightly packed in large numbers. Lymphatic nodules with germinal centers were not observed. Scattered diffusely throughout the lymphoid tissue were larger cells of reticuloendothelial type, with acidophilic cytoplasm and large oval or lobulated vesicular nuclei, frequently containing large, prominent nucleoli (fig. 2 C). Mitotic figures were noted in several of these cells. Infrequently, double or trinucleated forms were identified, measuring up to 15 microns in diameter. Larger forms were not encountered.

In areas, particularly where fibrosis was prominent, eosinophilic leukocytes were commonly noted, as many as 8 per high power field (objective, 4 mm.; ocular,  $\times 10$ ). The fibrosis and the eosinophilic and lymphoid infiltration combined to impart a distinct granulomatous appearance to these areas.

A similar infiltration was noted in the omentum.

The diagnosis submitted was lymphoblastoma of the ovary and the omentum, probably Hodgkin's type, with a notation that in all probability the involvement

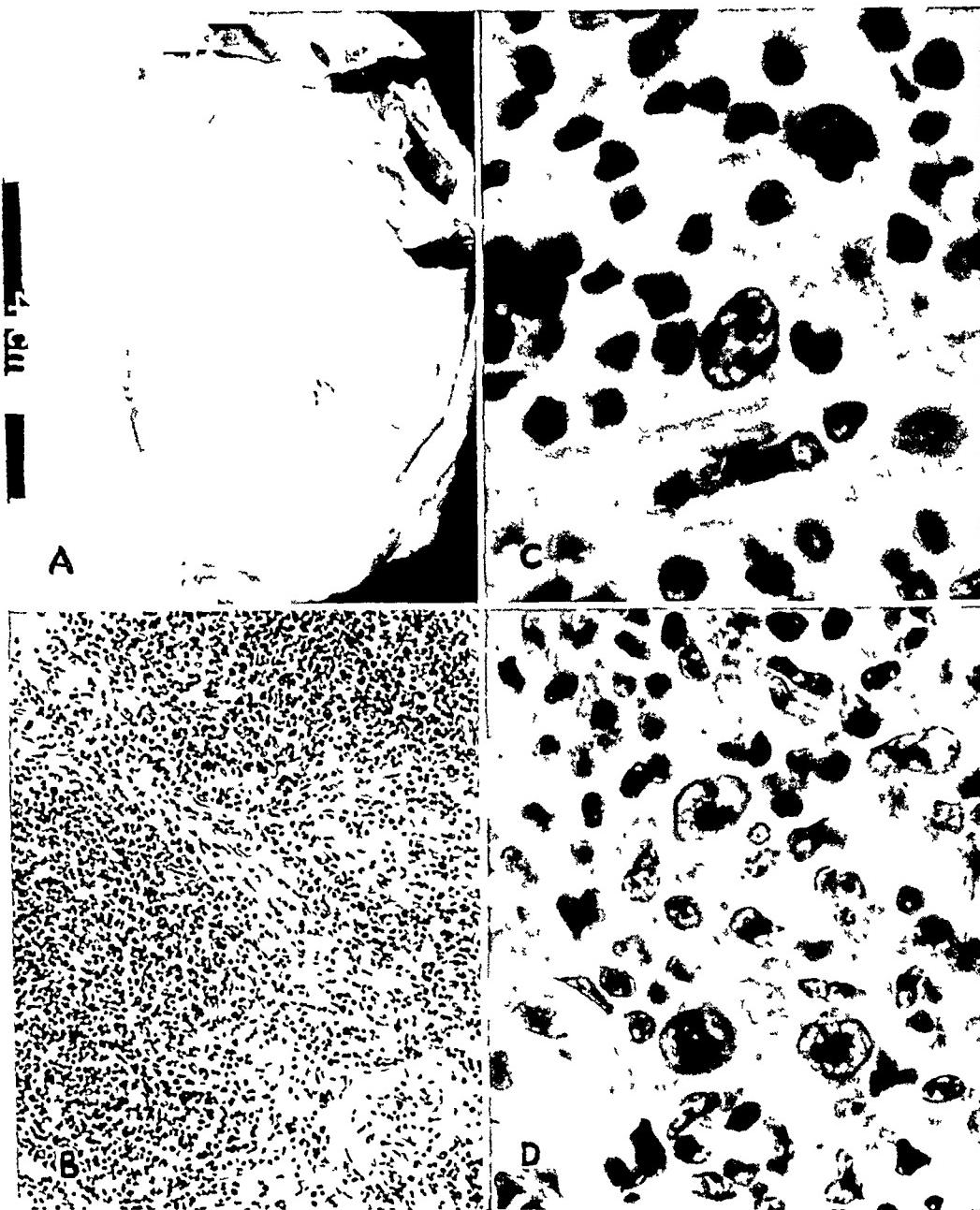


Fig. 2 (case 2).—*A*, ovarian tumor. The cut surface is pale, solid and homogeneous. The ovarian capsule covering the right surface is prominently displayed. Compressed ovarian parenchyma containing two cysts appears at the right upper pole.

*B*, ovarian tumor. The granulomatous appearance is evident.  $\times 130$ .

*C*, ovarian tumor. At the center is a large cell of the Sternberg-Reed type, with two prominent nucleoli. Numerous lymphocytes appear in the field.  $\times 1,000$ .

*D*, lymph node. Pleomorphism and fibrosis are distinct. In the central area there are three cells of the Sternberg-Reed type, one showing nuclear lobulation.  $\times 800$ .

of the ovary was secondary to a process primary in the abdominal lymph nodes. Following and presumably as a result of this report a discrete bean size subcutaneous nodule was detected in the right arm, overlying the area of the insertion of the deltoid muscle.

The patient was readmitted for excision of the subcutaneous nodule on July 17. In the meantime irradiation of the abdominal nodes had been instituted, and over a period of several weeks they showed considerable regression in size and were no longer palpable.

The specimen consisted of an oval encapsulated mass 2 cm. in length, to the surface of which small tags of fat were attached. The cut surface was pale, solid and glistening. The consistency was moderately firm. Sections revealed a lymph node showing moderately advanced alteration of the nodal structure. The germinal centers and the interstitial structure were almost completely replaced by a diffuse lymphoid infiltrate possessing no structural characteristics. The cells were relatively mature and tightly packed within a delicate connective tissue stroma. Scattered in small numbers throughout the pulp were reticuloendothelial cells possessing large oval or lobulated nuclei, generally vesicular but occasionally hyperchromatic and in mitotic division. Stromal proliferation had resulted in considerable thickening and lamination of the capsule, beyond which lymphoid infiltration of the perinodal fat was evident. In the peripheral areas larger reticuloendothelial cells of the Sternberg-Reed type were numerous. They were generally mononuclear (fig. 8).

#### COMMENT

The diagnosis of Hodgkin's disease in the 2 cases requires discussion.

In case 1 the lesions of Hodgkin's disease were of two types. Throughout the abdominal lymph nodes, which were regarded as the primary site, the picture was that of Hodgkin's sarcoma (Karsner<sup>6</sup>), with large irregular reticuloendothelial cells spreading diffusely throughout the interstitial pulp and sinusoidal spaces and exhibiting numerous mitotic figures. Pleomorphism was particularly pronounced. The second type was that designated as Hodgkin's paragranuloma (Jackson and Parker<sup>4</sup>), in which the infiltrate consisted of masses of closely spaced lymphocytic cells of mature type, containing few of the larger reticuloendothelial cells which characterize Hodgkin's disease. Both types formed the ovarian tumor. Exception may be taken to the diagnosis of Hodgkin's disease in this case inasmuch as the characteristic granulomatous reaction was not observed. Acceptance or rejection of the diagnosis depends entirely on one's opinion as to the necessary diagnostic criteria. If one accepts the broader concept of Hodgkin's disease as one with a variable pattern, subject to mutations and transition in which the tumor changes into the sarcomatous and almost pure lymphoid variants, which have been repeatedly described (Karsner; Ewing<sup>7</sup>; Jackson and Parker; Fabian, and others), the diagnosis in

6. Karsner, H. T.: Arch. Int. Med. 6:175, 1910.

7. Ewing, J.: Neoplastic Diseases, ed. 4. Philadelphia, W. B. Saunders Company, 1940, pp. 413-415.

this case becomes tenable. Special attention is directed to a study recently published by Herbut, Miller and Erf<sup>8</sup> which illustrates such variability of the histologic pattern in Hodgkin's disease.

In case 2 the ovarian infiltration was obviously lymphoid in character and not consistent with the pattern of any tumor primary in that organ. The occurrence of the larger cells of reticuloendothelial type, resembling poorly developed Sternberg-Reed cells, the scattered eosinophils and the fibrosis led to a diagnosis of lymphoblastoma, probably Hodgkin's disease. The reaction subsequently noted in the lymph node from the arm appeared to substantiate fully the original diagnosis.

That the ovarian involvement in both cases was not the result of fusion with, and direct extension from, contiguous lymph nodes was manifested by the fact that the organs were freely movable, in normal position, unattached to neighboring nodes and each surrounded by an intact ovarian capsule.

The identification of sarcomatous cells within the lumens of veins within the substance of the ovary in case 1 probably serves to explain the genesis of the infiltration in this organ.

#### SUMMARY

In 2 cases of Hodgkin's disease with unusual involvement of the ovary the gross appearance of the lesion was such that it was misinterpreted as a primary ovarian neoplasm.

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8. Herbut, P. A.; Miller, F. R., and Erf, L. A.: Am. J. Path. 21:233, 1945.

## PLASMODIUM FALCIPARUM MALARIA

The Coronary and Myocardial Lesions Observed at Autopsy in Two Cases of  
Acute Fulminating P. Falciparum Infection

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THE literature on malaria conveys the impression that the pathologic aspect of this disease is limited to splenomegaly and pigmentation of viscera, with the additional distinguishing feature of plugging of cerebral vessels and miliary necrosis in Plasmodium falciparum infection. Many authors interpret other lesions as caused by toxins liberated by the parasites or as the reaction from the anemia. What is it that predisposes to plugging of cerebral vessels, and why should one not anticipate a similar phenomenon in other vessels?

MacCallum<sup>1</sup> pointed out that coma in P. falciparum infection is not due to the focal necrosis but to the plugging of the cerebral capillaries. The question might be raised whether cerebral manifestations alone are sufficient to explain the sudden deaths of patients. Cecil<sup>2</sup> stated that a guarded prognosis must be held for a patient in coma or stupor with the cerebral type of P. falciparum malaria and that with hemoglobinuria the prognosis is always grave. Dudgeon and Clarke<sup>3</sup> are quoted: "Death from suddenly developed cardiac failure is common in pernicious forms of subtropical malaria and is due to the severe toxic fatty degeneration of the myocardium." Manson<sup>4</sup> reported gangrene of the toes; he pointed out that the defective nutrition, the edema of the ankles and the dilatation of the ventricles are due to anemia. Strong<sup>5</sup> mentioned fatty degeneration of the heart. Gaskill<sup>6</sup> reported the presence of parasites among and within the cardiac cells. Manson<sup>4</sup> included

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1. MacCallum, W. G.: Textbook of Pathology, ed. 7, Philadelphia, W. B. Saunders Company, 1940, p. 834.

2. Cecil, R. L.: Textbook of Medicine, ed. 6, Philadelphia, W. B. Saunders Company, 1943, p. 437.

3. Dudgeon and Clarke, cited by Strong,<sup>5</sup> p. 77.

4. Manson-Bahr, P. H.: Manson's Tropical Diseases, ed. 11, Baltimore, Williams & Wilkins Company, 1942, p. 71.

5. Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 7, Philadelphia, The Blakiston Company, 1944, vol. 1, p. 57.

6. Gaskill, cited by Strong,<sup>5</sup> p. 58.

cardialgia among the symptoms, and, crediting Dudgeon and Clarke,<sup>3</sup> attributed low blood pressure to thrombosis of vessels of the adrenal glands. Craig and Faust<sup>7</sup> mentioned no myocardial lesions. Most and Meleney<sup>8</sup> recently reported 7 cases of subtertian malaria, including 2 fatal cases, but in only 1 of these did they observe cerebral lesions. They made no reference to other organs.

It is probably an unusual experience to study the lesions of uncomplicated and untreated acute *P. falciparum* malaria. It might also appear strange that such cases should occur, particularly when the etiologic aspects are so well understood and routines for suppression and treatment so rigidly enforced. The fact remains that in spite of all precautions there will always be cases in which the infection follows a similar course.

The pathologic observations of these 2 cases should be of interest in that the mechanical factors of the cycle followed by the parasites in the blood undoubtedly contributed a great deal to the production of the symptoms and sudden death.

#### REPORT OF CASES

CASE 1.—A soldier aged 33, who had been on furlough, collapsed on the street in town. On admission to a hospital he was disoriented and could not give a reliable history. A blood smear was positive for *P. falciparum*, with 30 per cent of the red cells infested. His pulse was weak and rapid (rate, 112 to 140) but regular; the blood pressure was not recorded. The erythrocytes numbered 1,980,000 per cubic millimeter; the leukocytes, 20,600; the hemoglobin content was 47 per cent. The urine was dark brown and contained albumin (3 plus). His temperature did not rise above 101 F. Treatment was promptly instituted, but five hours after admission the patient died in a manner suggestive of embolism.

*Autopsy.*—The body was well developed and well nourished, with a tinge to the skin suggestive of quinacrine hydrochloride. The visceral cavities contained no free fluid. The heart was dilated, with the greatest transverse diameter 21 cm.; the weight was 400 Gm., and the musculature seemed very flabby, soft and dark. Small petechiae were seen in the epicardium; the coronary orifices, the valves and the endocardial surfaces were without change. The cut surface of the muscle was dark red but showed no evidence of infarction. The lungs were edematous. The spleen was a dark chocolate brown and weighed 570 Gm. The capsule was extremely thin and tense, and cracked readily when the spleen was manipulated. The cut surface also was dark chocolate brown; the pulp was soft and mushy and washed out readily. The stomach contained particles of undigested food, indicating that the man had a period of well-being just before his sudden collapse. The entire intestinal tract appeared normal. The liver was dark brown, dull and pasty, and weighed 2,020 Gm. There was no gross evidence of hemorrhage or necrosis. The kidneys weighed 280 and 300 Gm., respectively. The capsules were thin and tense and stripped with ease. The cut surfaces were reddish brown, and the cortex was sharply demarcated from the medulla. Grossly,

7. Craig, C. F., and Faust, E. C.: Clinical Parasitology, ed. 2, Philadelphia, Lea & Febiger, 1940, p. 204.

8. Most, H., and Meleney, H.: J. A. M. A. 124:71, 1944.

no hemorrhages were encountered. The brain showed no significant changes. There was not even pigmentation of the parenchyma. Other organs were considered normal.

*Microscopic Observations.*—(a) Heart: The muscle fibers presented a loose texture, and their striations were faint. In some areas the muscle sheaths were intact, but the cytoplasm was translucent, stained poorly and showed a flaky texture. Miliary areas of hemorrhage were also seen but no leukocytic reaction either in the ischemic or in the hemorrhagic areas. The vessels presented the most conspicuous feature; they either bulged or were plugged with parasites and parasitized red cells. Ameboid forms of parasites adhered to the walls of the vessels, and at the bifurcations clumps of ameboid forms plugged the lumens; frequently thrombi formed in these areas. Endothelial cells lining the capillaries were swollen, and some of them had assumed phagocytic function. Red cells containing trophozoites were seen among normal ones in the lumens of capillaries.

(b) Liver: The lobules were distinct, and only occasional red cells and parasites were encountered in the sinuses. The Kupffer cells were large and loaded with pigment granules. In the efferent zones the liver cells contained fat globules. There was also a moderate degree of passive congestion. Cloudy swelling was seen throughout.

(c) Spleen: The reticulum was engorged with red cells, many of which contained parasites. Large numbers of phagocytic cells contained debris and reddish brown pigment. Polymorphonuclears were relatively scarce. The malpighian bodies were compressed because of congestion. No scarring or hemorrhages were seen. The capsule was thin, and the trabeculations were delicate.

(d) Kidney: Except for swelling of the renal epithelial cells, which contained flaky cytoplasm, there were no significant changes. The capillaries throughout the cortex were relatively free from parasites. Toward the pelvis of the kidney the capillaries were engorged with parasitized red cells containing ameboid forms. There were no areas of necrosis.

(e) Adrenal Gland: The vascular sinuses were relatively free from parasites. There was no necrosis, and the cortical cells were well preserved.

(f) Bone Marrow; Rib: There was no evidence of destruction of the marrow. Except for occasional large macrophages containing pigment, the section showed no variation from the normal. There was no depletion of any of the myelogenous elements.

(g) Brain: The meninges had a loose edematous texture. The capillaries were engorged with red cells and parasitized cells. An occasional area of hemorrhage was encountered showing well preserved red cells and disintegration of the brain substance. There was no accumulation of leukocytes or glial cells in the vicinity of the hemorrhages. Parasitic thrombi or plugs were numerous.

(h) Lung: The sections showed a moderate degree of edema and marked engorgement of the vessels; many of the capillaries were conspicuous because of the parasitized red cells within. There was no pneumonia.

CASE 2.—A moderately obese man, 70 years of age, had been treated at a local dispensary for food poisoning because of indigestion and diarrhea. Six days later he lapsed into a semistupor and was sent to a hospital. On admission his temperature was 101 F. The pulse was regular and rapid; the rate, 110. Examination of the chest revealed moisture at the base of each lung; the heart was reported normal. The red cell count was 2,800,000; the leukocyte count was 16,500; the hemoglobin content was 50 per cent. A blood smear was positive for *P. falciparum*, 40 per cent of the cells being infested. The patient became coma

tose; the heart sounds were more distant; the pulse rate rose to 130 and was irregular and thready; the blood pressure was 60 systolic and 40 diastolic. Before transfusion and treatment were instituted, he suddenly became cyanotic and died, six hours after admission.

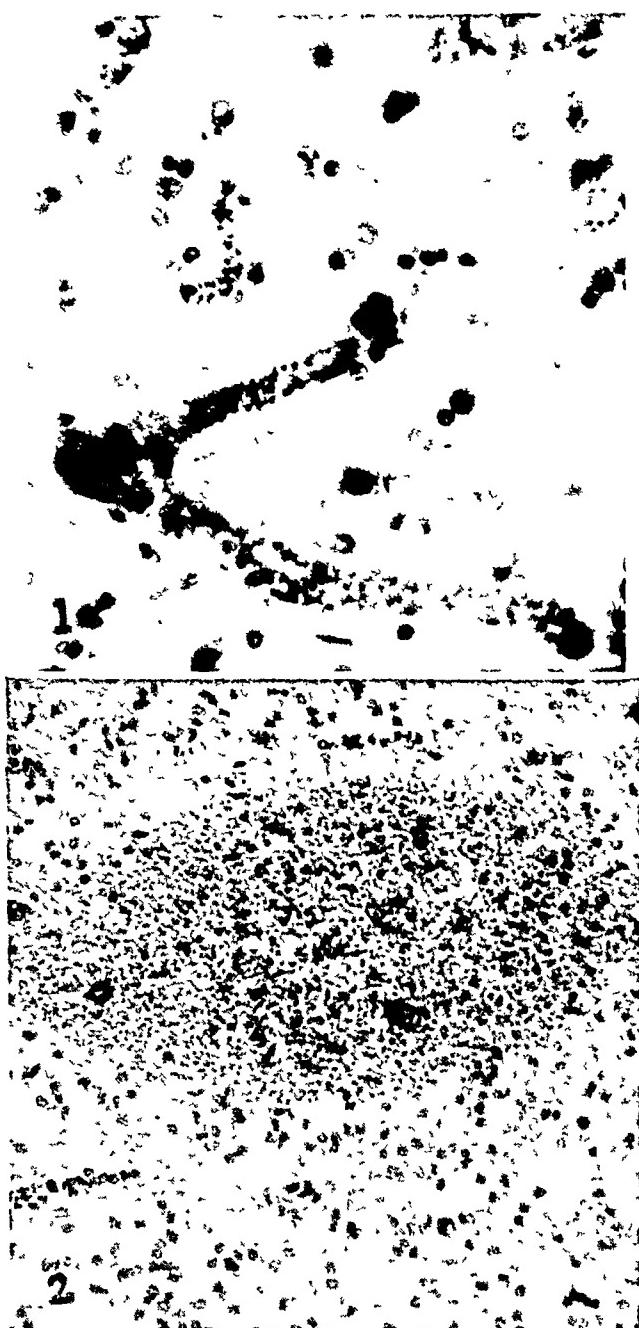


Fig. 1 (case 2).—Branching vessel of the brain, greatly dilated and plugged with ameboid forms of parasites and emboli.

Fig. 2 (case 2).—An area of fresh hemorrhage with cells well preserved and no increase of glial cells. Note capillaries occluded with parasitic emboli.

*Autopsy.*—The body was that of a moderately obese elderly white man. The visceral cavities were dry. The lungs were free from adhesions, had retracted and were air containing except along the posterior borders, where there was a moderate degree of congestion. The heart was dilated, particularly the auricles; the transverse diameter was 20 cm. The musculature was soft, and when the vessels were severed, the heart literally collapsed. The valves were all normal, and the coronary orifices were patent. On the epicardial surface there were dilated vessels but no scars or petechiae. The cut surface of the myocardium showed no gross evidence of hemorrhage or scarring.

The liver weighed 1,820 Gm., was reddish brown and of firm consistency; the cut surface looked parboiled, being a homogeneous reddish brown, with no evidence of hemorrhage or necrosis. The stomach and the entire intestinal tract were free from ulcerations and petechiae. The spleen weighed 740 Gm. and was dark red. The capsule was as thin as tissue paper and cracked under the slightest pressure. The pulp was soft, mushy, deep red and homogeneous. The left kidney weighed 210 Gm. and had a large cortical cyst at the lower pole; the right weighed 180 Gm. The cut surfaces showed a sharp line of demarcation between the cortex and the medulla. The capsules stripped with ease. There was no gross evidence of scarring. Atheromatous plaques were limited to the abdominal portion of the aorta. The meningeal vessels of the brain were engorged and resembled a red veil stretched over the cerebrum. The cerebral convolutions were flattened, and there was a moderate increase in the amount of fluid in the subarachnoid space. Cross sections through the hemisphere revealed normal color and no gross hemorrhage or pigmentation such as is described in chronic malaria. Organs not mentioned were without significant change.

*Microscopic Observations.*—(a) Brain: The outstanding feature was the marked engorgement of the capillaries with ameboid forms of parasites and parasitized red cells. Many of the capillaries were occluded and formed bulblike dilatations. The cells making up the endothelial lining of the vessels bulged into the lumens, and many contained pigment. Some of the vessels contained fresh thrombi. There were areas of petechial hemorrhages and necrosis, in most of which the red cells were well preserved. The capillaries in the spinal cord also contained clumps of parasitized cells. There was no leukocytic reaction.

(b) Adrenal Gland: The cortex was well preserved. There were no areas of necrosis or vacuoles, no engorgement of the sinuses with red cells or parasites, yet the vessels in the fat beyond the capsule were literally distended with plugs of parasites.

(c) Kidney: There was an occasional scarred glomerulus. The capillaries were relatively free from parasites except in the medulla. Epithelial cells appeared swollen and had pale flaky cytoplasm. There was neither necrosis nor infarction.

(d) Liver: The hepatic cells showed evidence of cloudy swelling. The Kupffer cells stood out conspicuously, saturated with a yellowish brown pigment. The sinuses were narrow and contained occasional red cells, some of which were parasitized. There was no fatty degeneration.

(e) Stomach and Intestinal Tract: The capillaries of the mucosae of the stomach and the entire intestinal tract were engorged and contained parasitized red cells, but there were no hemorrhages and no ulcerations.

(f) Spleen: The spleen appeared identical with that in case 1.

(g) Bone Marrow: The marrow was not hyperplastic, nor was there any imbalance in the types of cells. There was no necrosis and certainly no reservoir of parasites; in fact, the sinuses of the marrow contained fewer parasites than

most of the tissues. This was contrary to a current notion that the parasites collect in the marrow.

(h) Lung: There was no infection; the capillaries contained parasitized red cells but did not appear as if they were occluded. The picture was that of edema. There was no evidence of pneumonia.

(i) Heart: The capillaries were so distended and plugged with parasites and thrombi that some of them literally bulged. The larger vessels were empty, but rows of parasitized red cells containing ameboid forms adhered to the intima. The endothelial cells lining the capillaries were swollen, and many of them contained pigment granules. There were patches of myocardial degeneration; the cell walls were still intact, but the cytoplasm was translucent and hydropic. Even in the center of some of these areas the cell walls had not disintegrated. There was no proliferation of fibroblasts and no leukocytic invasion, all of which would indicate an ischemic type of infarction. The blocks taken near the apex of the heart showed more plugging of capillaries than those from near the base of the heart. The musculature had a loose texture, indicating edema. There was no evidence of coronary sclerosis.

#### COMMENT

The extremely rapid course of the disease and the briefness of the hospitalization in both of these cases precluded early diagnosis with institution of treatment. In case 1, although the diagnosis of *P. falciparum* malaria was made on admission, the patient, a comparatively young man, had already collapsed, and treatment was of no avail. Because of the advanced age of the patient in case 2, the malarial nature of the cardiac lesions was not suspected until too late; the recorded blood pressure of 60 systolic and 40 diastolic, so usual in cardiac failure, served further to obscure the diagnosis. Death in both cases was typical of cardiac dilatation and collapse. Neither patient was in actual coma, but both of them seemed drowsy, even though they could be aroused.

One would experience considerable difficulty in distinguishing the types of malaria on the basis of the gross findings at autopsy except for the dilated heart with dark, reddish, soft musculature found in both of these cases, proved to be cases of *P. falciparum* malaria. It is not until the tissues are examined microscopically that the characteristic lesions of this type of malaria become unmistakable. My chief reason for selecting these cases is that the photomicrographs made illustrate these changes so closely.

Plugging of cerebral vessels has been demonstrated repeatedly and is always emphasized in discussions of the pathologic aspects of *P. falciparum* infection. One should not overlook the fact that the coronary system is as terminal in character as is the circulation of the brain and has as poor a collateral supply. Therefore, one should expect to find parasitic plugging or even thrombi in the coronary vessels as well as in those of the brain. It is only natural that the occlusion should begin in the most distal branches, the caliber of which is small, since the

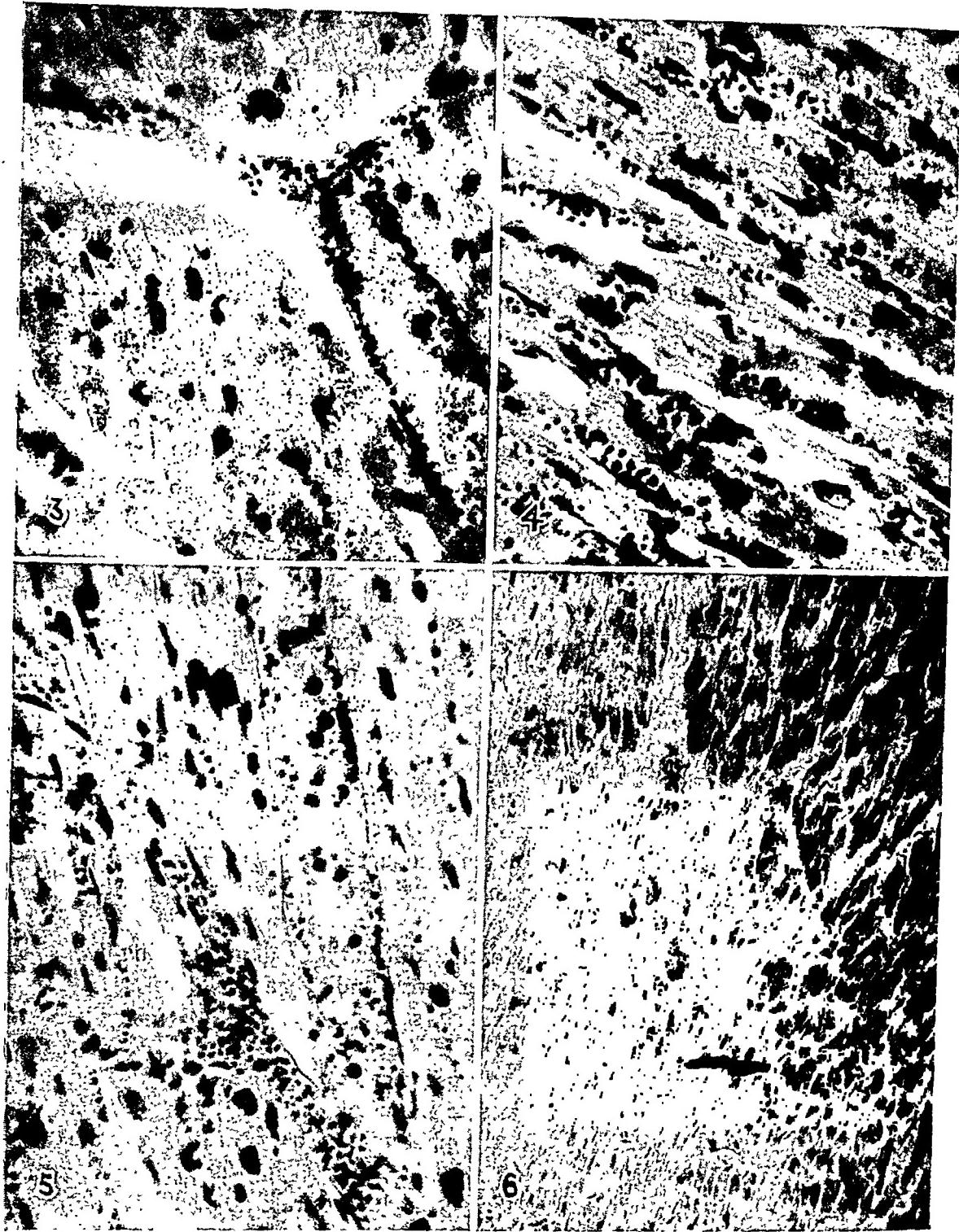


Fig. 3 (case 1).—Heart muscle showing a branching vessel occluded with parasites. In the large branch the parasites cling to the wall. The red cells free in the lumen contain relatively few parasites.

Fig. 4 (case 2).—Myocardium in acute *P. falciparum* malaria. All the capillaries are occluded with parasites. The endothelial cells lining capillaries are swollen, the muscle fibers have lost their striations, and early hydropic degeneration is present.

Fig. 5 (case 2).—Myocardium showing distended capillaries plugged with parasites. In the larger vessels rows of parasites adhere to the walls.

Fig. 6 (case 2).—Myocardium with a large area of degeneration. Note the absence of fibrosis. Many muscle fibers in the degenerated area still retain intact cell walls. Capillaries plugged with parasites are seen along the perimeter of degeneration.

process is not unlike embolism, even to having the same patchy distribution. The interval between occlusion and death may be so short that no discoloration occurs and grossly the heart wall shows no indication of the area involved, a situation which makes the selection of blocks difficult. I have found that the blocks taken from areas equidistant from the two coronary arteries and near the apex are most apt to show the lesions. Blocks should be from 1 to 2 cm. in thickness; otherwise the fixing fluid will wash out the engorgement and may even release the fresh plugs. These blocks should be fixed for periods of from twelve to twenty-four hours and then may be cut down to the proper size for complete fixation.

The striking tendency of the parasites and parasitized red cells to adhere to the capillary walls is clearly seen in the illustrations, and only the ameboid forms are lined along the inner surface of the vessels (fig. 3). The endothelial cells of the capillaries are swollen and often contain phagocytosed malarial pigment. I have not been able to demonstrate a similar phenomenon for *Plasmodium vivax* infections.

The lesions in the heart resemble infarcts in that they are rather sharply demarcated. Throughout them it is interesting to find the capillaries plugged with parasitized red cells, but there is neither hemorrhage nor leukocytic reaction, and the cell walls of the muscle fibers are intact. The areas are conspicuous because they stain poorly with hematoxylin and eosin and show no fat globules with fat stains; furthermore, all striations of the included muscle fibers are obliterated, and the cytoplasm is hydropic and flaky. These areas may be recent, and it is possible that after the segmentation of the parasites the circulation is resumed in the channels where the occlusions have not developed into thrombi, and the muscle fibers are restored to normal.

During the time that these occlusions exist, the organ must experience anoxemia proportional to the extent of the area involved. Clinically, therefore, the condition is like any other form of coronary occlusion, and the fatality in malaria due to *P. falciparum* will depend on the degree of occlusion and any added strain of the heart during the course of treatment.

I have included illustrations which show cerebral lesions and plugging of vessels. The marrow showed no change and no parasites, which contradicts the old theory that the marrow acts as a reservoir for the parasites. In the liver and the adrenal gland, the sinusoids were not clogged with parasites, and there was no tendency for the parasitized cells to cling to the vessel walls. The spleen appeared the same as in *P. vivax* infections.

Microscopic evidence indicates that the coronary occlusions producing anoxemia of the heart may be as important a factor in fatal cases of *P. falciparum* malaria as the occlusions of cerebral vessels.

## SUMMARY

Ameboid forms of *Plasmodium falciparum* have a tendency to adhere to all capillary walls; consequently they may bring about occlusion, plugging or thrombosis. The progress of events indicates that this parasite may produce symptoms because of these mechanical factors. The coronary system of the heart offers a poor collateral circulation; hence plugging of coronary vessels is apt to create anoxemia of the myocardium, resulting in dilatation and collapse.

## RETICULUM

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RETICULUM was first observed by Kupffer<sup>1</sup> in the liver and was described by him as a fibrillar network ramifying throughout the organ on the sinusoidal surfaces of the hepatic cords in close association with the stellate cells which bear the discoverer's name. Mall<sup>2</sup> distinguished reticular from collagenous and elastic fibers. He observed that the former were comparatively resistant to acid and alkaline solvents and digestive ferments and that they were made relatively distinct by such digestive preparation. Russakoff,<sup>3</sup> Kon,<sup>4</sup> Rössle and Yoshida<sup>5</sup> and others described reticulum in various organs throughout the body, including the liver, the kidney, the pancreas, the lymph nodes and the lungs. Reticulum as a product of granulomatous inflammatory processes, particularly in the presence of tuberculous infection, was described by Miller<sup>6</sup> and by Foot.<sup>7</sup> Studies of the histogenesis of reticulum were reported by Corner,<sup>8</sup> Foot,<sup>9</sup> Mallory and Parker,<sup>10</sup> Rinehart<sup>11</sup> and others, and the reticulum occurring in tumors was discussed by various contributors, notably White,<sup>12</sup> Niosi,<sup>13</sup> Foot and Day<sup>14</sup> and Mallory and Parker.<sup>10</sup> Their observations, as well as the various contributions to technical methods, will be referred to later.

During the past five years, my associates and I have prepared histologic sections of pathologic tissue for the demonstration of reticulum

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From the Department of Pathology, Indianapolis City Hospital.

1. Kupffer, C.: Arch. f. mikr. Anat. **12**:351, 1876.
2. Mall, F. P.: Abhandl. d. math.-phys. Cl. d. k. sächs. Gesellsch. d. Wissenschaften **17**:299, 1891; Johns Hopkins Hosp. Rep. **1**:171, 1896.
3. Russakoff, A.: Beitr. z. path. Anat. u. z. allg. Path. **45**:476, 1909.
4. Kon, Y.: Arch. f. Entwicklungsmechn. d. Organ. **25**:492, 1908.
5. Rössle, R., and Yoshida, T.: Beitr. z. path. Anat. u. z. allg. Path. **45**:110, 1909.
6. Miller, W. S.: Am. Rev. Tuberc. **7**:141, 1923.
7. Foot, N. C.: Am. J. Path. **1**:341, 1925.
8. Corner, W. G.: Contrib. Embryol. **9**:85, 1920.
9. Foot, N. C.: Am. J. Path. **3**:401, 1927.
10. Mallory, F. B., and Parker, F., Jr.: Am. J. Path. **3**:515, 1927.
11. Rinehart, J. F.: Am. J. Path. **6**:525, 1930.
12. White, W. C.: Bull. Johns Hopkins Hosp. **11**:209, 1900.
13. Speciale, F.: Tumori **10**:37, 1923-1924.
14. Foot, N. C., and Day, H. A.: Am. J. Path. **1**:681, 1925.

almost as a routine. It is the purpose of this report to summarize the experience thus accumulated, together with that previously made available by the aforementioned workers, regarding technical methods, the nature and the histogenesis of reticulum, its appearance in pathologic lesions, and particularly the practical importance of reticulum, such as it may be, in pathologic diagnosis.

#### TECHNICAL METHODS FOR THE DEMONSTRATION OF RETICULUM

The study of reticulum was stimulated greatly by the discovery of Maresch<sup>15</sup> that the ammoniacal silver nitrate solution originally devised by Bielschowsky<sup>16</sup> for impregnation of neurofibrils would demonstrate reticular fibers. Improvements were made chiefly by Perdrau,<sup>17</sup> who introduced the preliminary procedure of treating sections with potassium permanganate, Laidlaw,<sup>18</sup> who suggested that oxalic acid be used to secure additional reduction, following the usual toning with gold chloride, and Foot,<sup>19</sup> who provided more complete differentiation through addition of Van Gieson's trinitrophenol and acid fuchsin. Various modifications have been offered, and we have used all of them more or less. The method which we have found most serviceable is based on the principles laid down by the aforementioned contributors, with some modification of Masson's methods<sup>20</sup> being used when counterstaining is desired; it is essentially as follows, subject to modification according to individual taste and the variability of action of different samples of chemical reagents.

1. Fix tissue overnight or longer in solution of formaldehyde U. S. P. (1:10), dehydrate with any standard reagent and embed in paraffin. Cut sections at 6 to 8 microns. With abundant thick egg albumin containing a minimum of glycerin, fix sections to slides carefully kept free of paraffin and grease. In spreading the albumin, a finger should be used which does not come in contact with paraffin on handling blocks, and the albuminized slide should not come in contact with water until the section is ready to mount. Dry sections in an oven at 37 C. at least overnight, and for two days if time is allowed.
2. Deparaffinize and carry sections to water in the usual manner.
3. Place sections for five minutes in 0.25 per cent potassium permanganate.
4. Rinse in water.
5. Place in 2 per cent oxalic acid until colorless.
6. Wash in water, preferably running, for five minutes.

15. Maresch, R.: Centralbl. f. allg. Path. u. Anat. **16**:641, 1905.

16. Bielschowsky, M.: Arch. f. Psychiat. **39**:1321, 1905.

17. Perdrau, J. R.: J. Path. & Bact. **24**:117, 1921.

18. Laidlaw, G. F.: Am. J. Path. **5**:239, 1929.

19. Foot, N. C.: J. Lab. & Clin. Med. **9**:777, 1924.

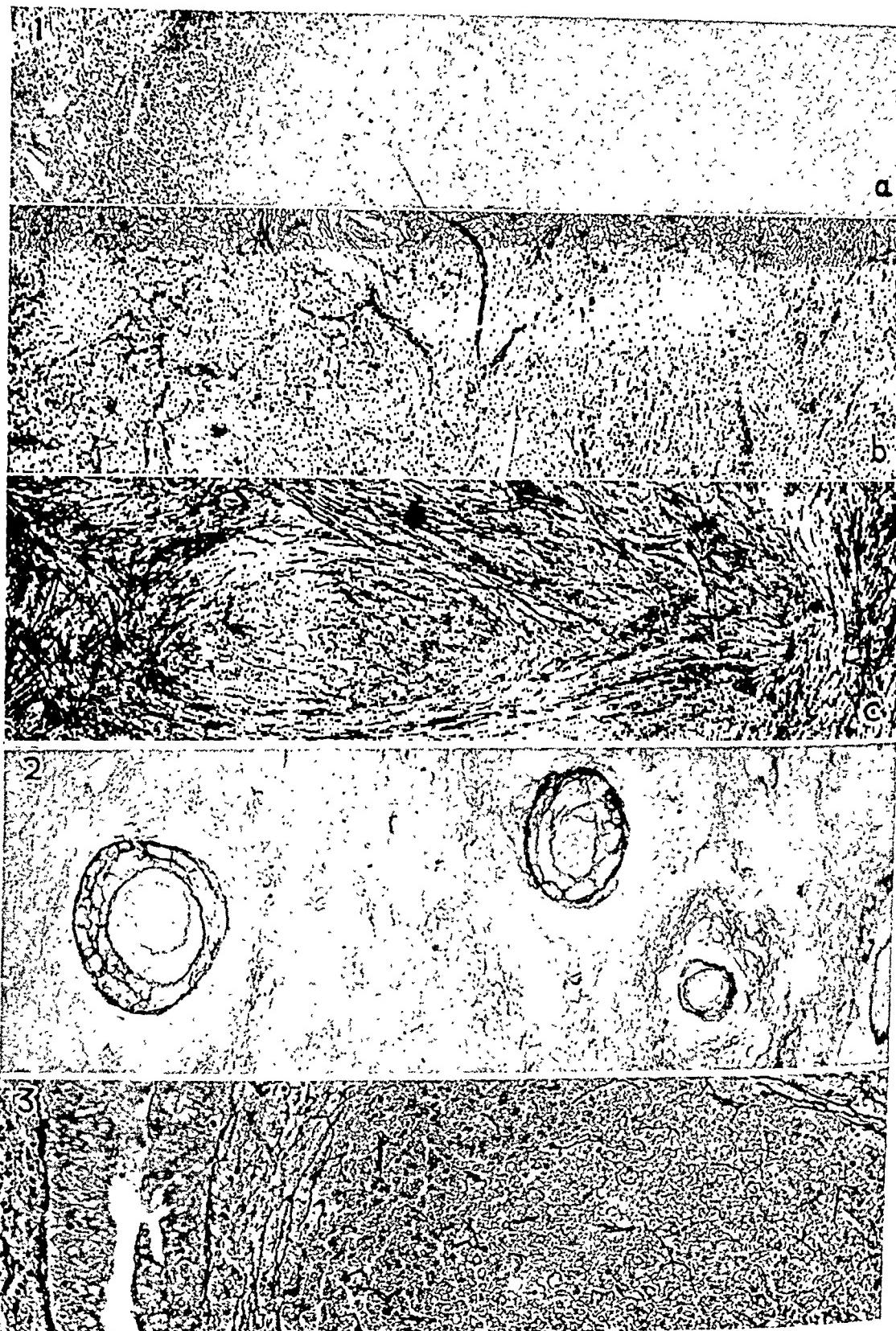
20. Masson, P.: J. Techn. Methods **12**:75, 1929.

7. Place for two minutes in each of two changes of distilled water.
8. Place for forty-five minutes in 10 per cent silver nitrate.
9. Rinse thoroughly in two changes of distilled water; for example, wave the slide back and forth ten times in the first jar and thirty times in the second.
10. Place for thirty minutes in ammoniacal silver nitrate solution, made up as follows: Take 15 cc. of 10 per cent silver nitrate from the bottle used for step 8. (This will use up the solution at a rate sufficiently rapid to insure adequate freshness of silver nitrate solutions.) To this, add ammonia, one-third strength, drop-wise but rapidly until the precipitate is nearly dissolved. The reaction is relatively slow, and the effect must be observed for at least thirty seconds before it is accepted as final. For the sake of economy of time, however, one may be bold in adding ammonia, as a small amount of silver nitrate can be added again if all the precipitate is dissolved. All that matters is that a few black grains remain on the filter paper. Filter the solution into the staining jar and, if necessary, add distilled water to bring the fluid level high enough to cover the sections. Mounting the section toward one end of the slide will promote economy.
11. Rinse as in step 9.
12. Place for five minutes in neutral solution of formaldehyde U. S. P. (1: 10).
13. Wash thoroughly.
14. Place in 0.3 per cent gold chloride for three minutes or until sections turn gray.
15. Rinse in distilled water.
16. Place in 2 per cent oxalic acid for five minutes or until sections turn purple.
17. Rinse in distilled water.
18. Place for three minutes in 5 per cent sodium thiosulfate ("hypo").
19. Wash, preferably in running water, for five minutes.

If no counterstaining is desired, sections may be dehydrated and mounted by any standard method. If counterstaining is desired, the following additional steps may be carried out:

20. Place for five minutes in a mixture of equal parts of 1 per cent phloxine and 1 per cent ponceau 2R (called xylidine ponceau in the Masson technic) containing 0.1 per cent acetic acid.
21. Rinse briefly in 0.1 per cent acetic acid.
22. Place for fifteen seconds to five minutes in a solution containing 2 per cent orange G and 5 per cent phosphotungstic acid.
23. Rinse briefly in 0.1 per cent acetic acid.
24. Place for two minutes in 0.1 per cent light green containing 0.1 per cent acetic acid.
25. Rinse briefly in 0.1 per cent acetic acid.
26. Rinse briefly in 95 per cent alcohol (until the green ceases to run rapidly). Dehydrate for eight minutes in acetone, clear in xylene for ten minutes and mount in balsam or gum dammar. Results: reticulum black, nuclei gray, collagen green, cytoplasmic structures various shades of red to orange or brown.

*Comment.*—(a) Fixation: Repeated trial has proved to our satisfaction that solution of formaldehyde U. S. P. is the fixative of choice for reticulum. Reagents containing mercury, chromium or other heavy metals, while rendering very sharp the detail of collagen fibrils after the use of aniline blue, seem to us to produce



(See legend on opposite page)

relatively unprecise definition of silver-staining fibers, whether reticular or axonal; solution of formaldehyde U. S. P. yields adequate fixation of the most delicate fibrils. It will accordingly be found that the preparation of sections to show reticulum can be carried out with particular convenience in laboratories employing solution of formaldehyde U. S. P. as the routine fixative.

(b) Nature of the Silver Nitrate Solution and Its Relation to Loss of Sections from Slides: After considerable trial of different forms of ammoniacal silver nitrate solution, such as those prepared with lithium carbonate, sodium carbonate and sodium hydroxide, we are convinced that while the carbonate solutions may render a slightly heavier or more detailed impregnation, the difference between the results from carbonated and those from purely ammoniacal solutions is so slight as to be detected with difficulty in unlabeled serial sections prepared with the two methods. That this difference is of value in research may be questioned, and it certainly may be disregarded in routine pathologic practice. The alkali tends, in spite of tedious repeated washing of the precipitated silver carbonate or hydroxide in an attempt to wash out excess alkali, to digest the albumin and detach the sections from the slides. If, on the other hand, the slides are kept free of paraffin and grease by the precaution mentioned previously, if thick albumin is used generously and is not washed off prematurely, if the sections are dried overnight or, if possible, for two days, and if ammonium silver nitrate is used in the concentration given, failure of impregnation and loss of sections will occur rarely.

(c) Mordanting: I have failed to find anything better than the Mallory "bleach," applied by Perdrau to reticulum. Immersion in 10 per cent silver nitrate for forty-five minutes will be found adequate for intermediate mordanting or sensitizing. The use of ferric alum, recommended by Gomori,<sup>21</sup> and other reagents results, in our hands, in little improvement and frequently renders a picture in which the tissue as a whole, including reticulum, lacks the richness of texture produced by Perdrau's method.

(d) Counterstain: It will be found that the Perdrau technic, followed by reduction of gold with oxalic acid as suggested by Laidlaw, yields a picture requiring little counterstain and serving most routine laboratory needs. Collagen fibrils are rose colored or brown and are fairly easily distinguished from reticulum. In routine practice one wishes to know, not how much reticulum there is in proportion

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#### EXPLANATION OF FIGURES 1, 2 AND 3

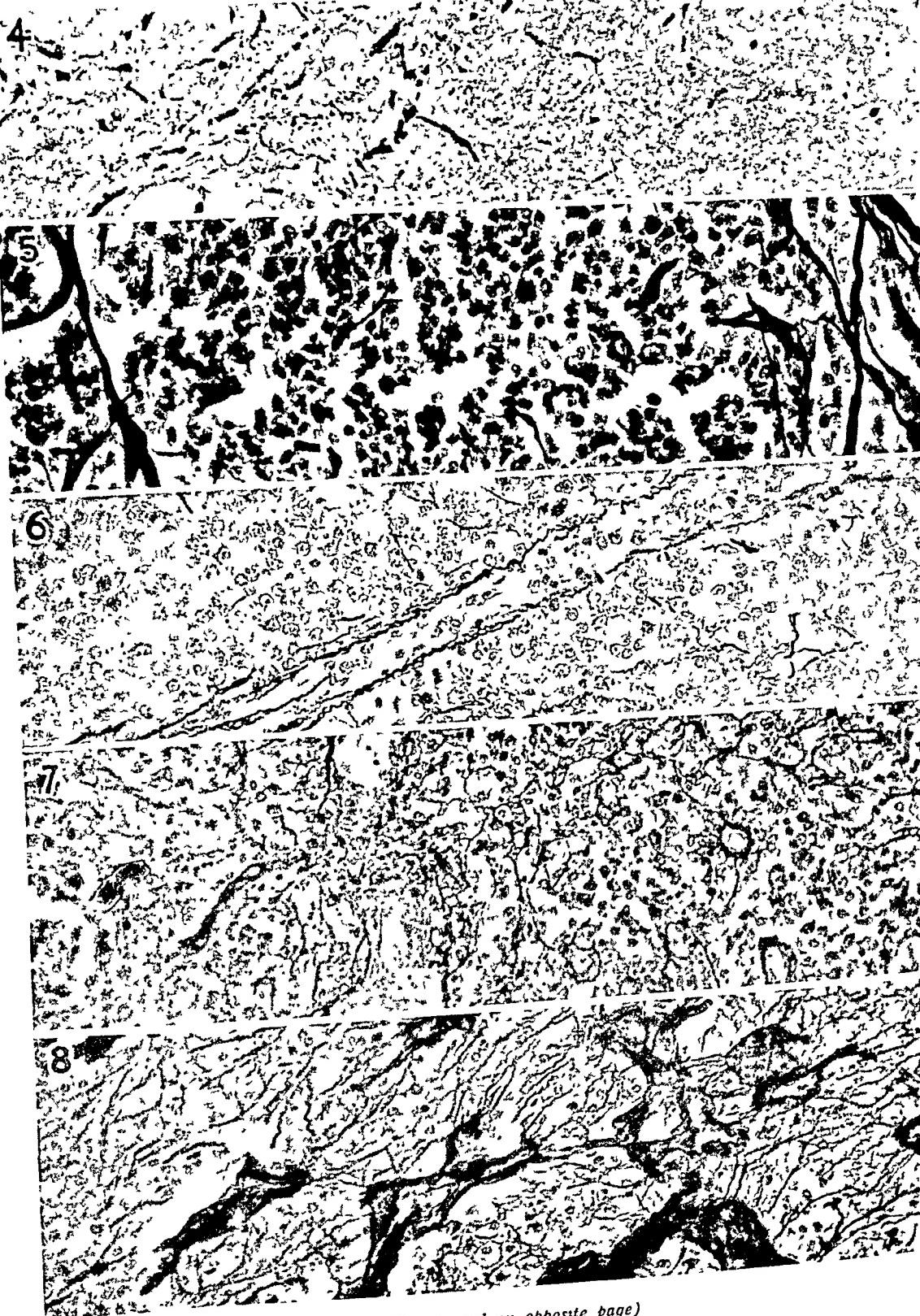
Fig. 1.—Tuberculosis of a hilar lymph node: (a) Border of a necrotic area; hematoxylin and eosin;  $\times 125$ . (b) The same field; Perdrau's method;  $\times 133$ . There is a slight rotation of the section on the slide compared with a. (c) Another field in the same section; Wilder's method;  $\times 278$ . This shows the characteristic tuberculoid pattern of reticulum at the site of a tubercle. When prepared with hematoxylin and eosin, this whole field was completely lacking in detail.

Fig. 2.—Section from a gumma of the brain; Perdrau's method;  $\times 267$ . It shows the characteristic lamellar reduplication of reticulum about the narrowed lumens of blood vessels. All tissue of this section was largely lacking in detail when prepared with hematoxylin and eosin.

Fig. 3.—Adenoid tissue; Laidlaw's method;  $\times 178$ . It shows absence of reticulum in the modified squamous epithelial lining of a crypt. Part of a follicle is seen; in it the reticulum is relatively sparse.

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21. Gomori, G.: Am. J. Path. 15:493, 1939.



(See legend on opposite page)

to collagen, but simply what the total reticular pattern is; i. e., is there or is there not reticulum between the tumor cells? Inasmuch as collagen has essentially the same diagnostic significance, confusion of collagen with reticulum will not matter greatly. One cannot expect, however, to show collagen as such brilliantly with an uncounterstained section prepared for reticulum. It will be found that the more delicate the counterstain the clearer the reticular pattern is. It is, for example, very easy to override the section too darkly with aniline blue. For this reason, a stain for Nissl substance is useful, as the thionine or the cresyl violet will enhance the nuclear pattern but will leave the network relatively bare. (Take sections previously stained for reticulum and immerse in 1 per cent aqueous thionine or cresyl violet for five minutes; rinse in water, rinse quickly in 95 per cent alcohol, dehydrate five minutes in absolute alcohol, clear in xylene and mount in balsam or its equivalent.) Masson's light green is an admirable collagen stain because it is reasonably intense and specific but treats fibrils with comparative delicacy and does not "steal from" reticulum as aniline blue frequently does. The use of the red and orange ingredients is more or less effective in completing the tissue detail. It will often be found that cytoplasmic and intercellular structures, collagen perhaps excepted, do not stain so well after the reticulum method has been used as after staining with hematoxylin. This is not so much the case after the use of methods for axis-cylinders, in which event, for example, healthy myelin can be stained orange quite faithfully. It is, in fact, advantageous to stain serial sections for reticulum and for nerve fibers; all one has to do is to prepare one of the sections by passing it through steps 4 to 7 while the other stays in water, and then carry both together through the remainder. This will yield the highly effective results of combined staining of nerve tissue<sup>22</sup> and rather complete staining of reticulum. To obtain certain varied results, a third section can be prepared by Bodian's method<sup>23</sup> as far as reduction of the protein silver with hydroquinone. The section may then be placed in water (step 13) and carried the rest of the way with the other slides.

(c) Cumbersomeness of the Method: Preparation of sections for the demonstration of reticulum in the busy laboratory may well be viewed with skepticism because

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#### EXPLANATION OF FIGURES 4 TO 8

Fig. 4.—Lymphosarcomatous infiltration of skin; Perdrau's method;  $\times 273$ . Scarcely any reticulum is seen except about vessels and in the main connective tissue bundles.

Fig. 5.—Lymphosarcoma of a lymph node; Perdrau's method and thionine,  $\times 318$ . There are foci relatively free of reticulum. Thick bands are stained, as well as fibrils.

Fig. 6.—Lymph node; Perdrau's and Masson's methods;  $\times 273$ . A diagnosis of reticulum cell sarcoma was made by three experienced pathologists, our own opinion not being represented in the diagnosis. Reticulum is relatively sparse.

Fig. 7.—Brain showing perivascular' sarcomatous proliferation of reticuloendothelial type; Laidlaw's method and thionine,  $\times 227$ .

Fig. 8.—Hemangioblastoma of meninges; Laidlaw's method and thionine;  $\times 227$ .

22. Dublin, W. B.: J. Neuropath. & Exper. Neurol. 2:205, 1943; Arch. Dermat. & Syph. 50:361, 1944.

23. Maximow, A. A. and Bloom, W.: A Text-Book of Histology, Philadelphia, W. B. Saunders Company, 1930, pp. 95-96.

of the time and the labor required for its performance. It will be found, however, that by devoting a corner of a cupboard to a permanent row of staining jars containing the appropriate solutions, together with accessories, such as a small flask for preparing the ammoniacal silver nitrate solution and a dropping bottle for ammonia, labor is reduced to a minimum. The silver nitrate (if any of this is left in the staining jar, keep the cupboard closed to exclude light), the solution of formaldehyde U. S. P., the gold, the oxalic acid and the sodium thiosulfate may be used for a surprisingly long time. The jars for rinsing in distilled water, if kept

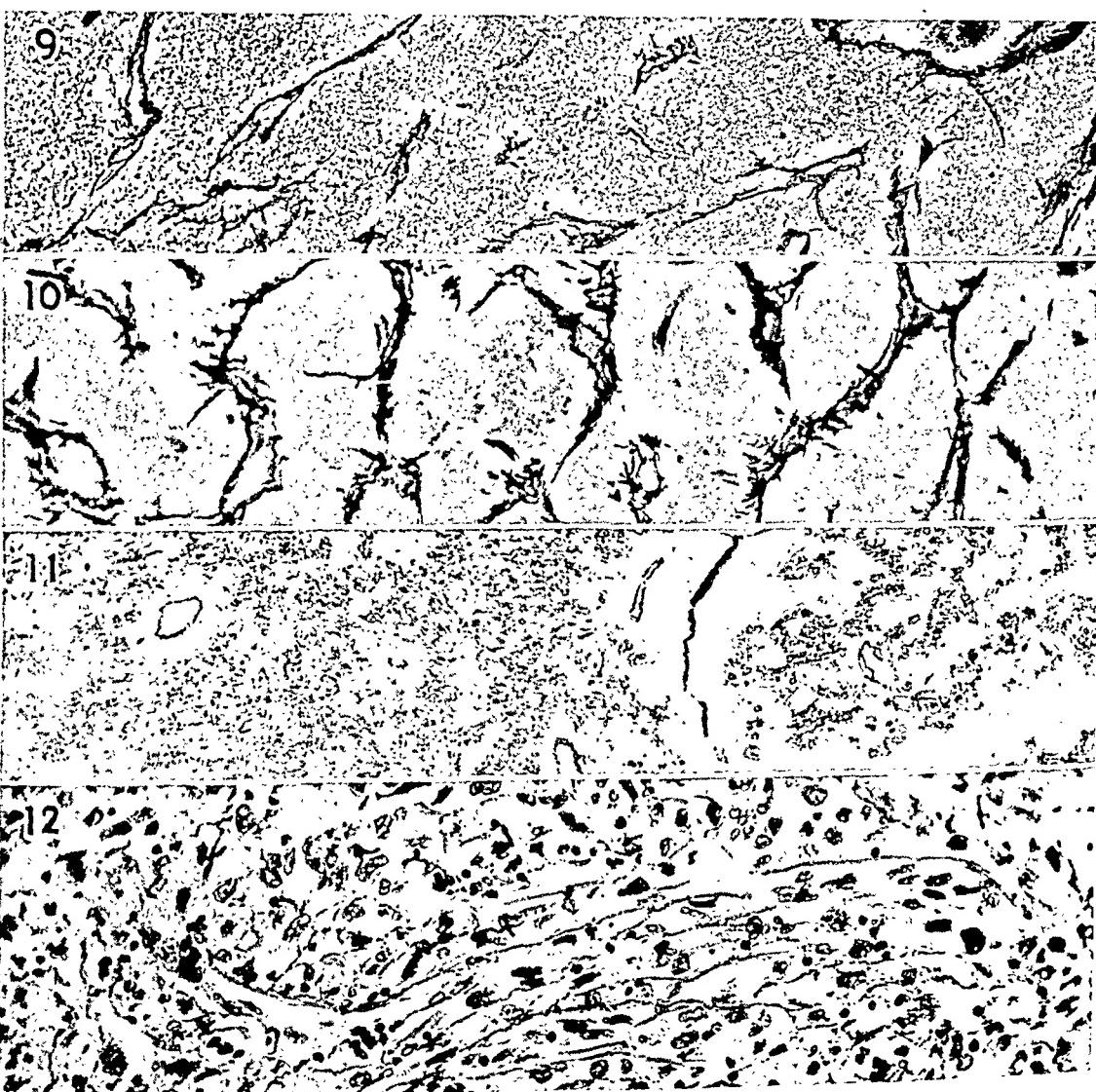


Fig. 9.—Neuroblastoma showing no intercellular reticulum; Laidlaw's method;  $\times 130$ .

Fig. 10.—Ewing's tumor showing no intercellular reticulum; Perdrau's method;  $\times 174$ .

Fig. 11.—Granulosa cell tumor showing absence of reticulum except for stroma and blood vessels; Perdrau's and Masson's methods;  $\times 130$ .

Fig. 12.—Hypernephroma, in which both the histologic pattern and the reticulum simulate sarcoma; Perdrau's and Masson's methods;  $\times 261$ .

permanently in place, need only be emptied. (Caution: The jar used for first rinsing after sections have been removed from the ammoniacal silver nitrate solution, the flask used for making up the ammoniacal silver nitrate solution and the jar used for staining with that solution should be rinsed out carefully to avoid the gradual production of silver fulminate, with the danger of explosion later on.) If to the foregoing one adds the custom of running ammonia in rapidly, adding a small excess of silver nitrate if need be, one has a staining routine for reticulum and for nerve tissue which is facile and reliable enough to meet the demands of any laboratory, however busy, which has a place for the use of essential special methods in histologic diagnosis.

#### NATURE OF RETICULUM

It seems to be pretty well agreed that connective tissue fibers may be recognized in three forms: collagen, reticulum and elastin. Reticulum and collagen have properties which differ in some respects, although having in the main a close affinity. There is some difference of opinion on this last-mentioned subject (relation of reticulum to collagen), the views being that: (1) reticulum and collagen are one and the same,<sup>10</sup> (2) reticulum and collagen have the same chemical properties but have slight differences of physical state and (3) reticulum and collagen differ physically and chemically. Maximow and Bloom<sup>23</sup> stated that although collagenous fibers, in the general opinion, do not branch, reticular fibers do. The first fibers to appear when collagenous tissue is formed in the embryo or in the adult body are argyrophil networks; these are gradually transformed into collagenous bundles. The methods generally used for the demonstration of collagenous fibers do not stain reticulum distinctly. Mall<sup>2</sup> showed by digestion experiments that reticulum is comparatively resistant to acid and alkaline solvents and digestive ferments. Foot<sup>1</sup> made particular use of his differential staining method in demonstrating that reticulum appears to be a precursor of collagen and that its fibers are gradually collagenized, in other words, that reticulum is precollagen. A study especially of tuberculous lesions leaves one with an impression that this relation of reticulum to collagen is amply supported. The argentophilic fibers can be seen to pass almost imperceptibly into the developing collagenous tissue. Depending on whether acid fuchsin, light green or aniline blue is used, varying amounts of tissue give the staining reaction for collagen and for reticulum. It is of note that acid fuchsin, used to stain collagen in the Van Gieson method, may be used in the modified Masson method, yet will yield to the stronger effect of light green or aniline blue.

Further than the foregoing statements there is little of a definite or proved nature that can be said of the chemical or the physical properties of reticulum. Mallory and Parker<sup>10</sup> reached the conclusion that reticulum and collagen are identical as a result of their observation that

when certain connective tissue fibers are found singly they give the staining reaction for reticulum but when they join a bundle of fibers they appear as collagen. That this view is open to question can be shown easily by use of a combined staining method. Separate fibers will, in a section stained in this way, commonly appear blue (or green) or black, and different portions of the same fiber may appear irregularly blue or black. It will further be found that thick fibers or bundles of fibers, apparently homogeneous when prepared routinely, frequently show a heterogeneous mixture of blue and black fibers when stained for reticulum and collagen. This is particularly true of the collagenous, argentophilic bands seen in the central or fibrous portions of rheumatic nodules. Broad bands of fibrous tissue when stained specially will frequently stain entirely black. We therefore take the view that reticulum is a precursor of collagen and is quite similar to, but chemically and physically different from, collagen.

#### HISTOGENESIS OF RETICULUM

Differences of opinion concerning the method of the formation of reticulum are bound up to some extent with factors referred to in the preceding paragraph. Mallory and Parker,<sup>10</sup> for example, expressed the belief that reticulum is produced only by fibroblasts, but this belief is confronted with the same difficulties which face the concept of identity of collagen and reticulum. That reticulum and collagen are produced by fibroblasts cannot be denied, for pure fibroma possesses abundant reticulum. So also, however, do pure collections of epithelioid cells or of lymphocytes, and it seems necessary, therefore, to accept the view that reticulum originates from all the aforementioned types of cells. It is well to bear in mind also that reticular cells, precursors of fibroblasts, are commonly found in association with collections of fibroblasts. This can be shown with Hortega's methods as well as with certain routine ones.

There seems to be little question that reticulum can be laid down by perithelial reticuloendothelial cells and that it ramifies freely in the various layers of the vessel wall, outlining the intima clearly. Whether reticulum is formed by vascular endothelium (excluding that of lymph node sinuses) and/or connects directly with endothelial cells is not agreed on. Corner<sup>8</sup> and Rinehart<sup>11</sup> demonstrated to their satisfaction that reticular fibers arise in, extend from and are in direct connection with vascular endothelial cells. Foot,<sup>24</sup> on the other hand, was unable to demonstrate that reticular fibers connect directly with cell bodies in the way that dendrites join the cell bodies of neurons. Foot con-

24. Foot, N. C.: Am. J. Path. 3:401, 1927.

sidered reticulum to be the precipitated form of an intercellular substance secreted by reticuloendothelial cells and viewed collagen as a chemical substance in tissue juices that impregnates fibers, rather than as the sole constituent of white fibrous tissue.

I have made no special study of the problem of the histogenesis of reticulum but have gained the impression that it originates from multiple cell types, as explained previously. I have also received the impression that reticular cells and reticulum have no definite connection, the cells lying along the fibers and on occasion rounding up and detaching themselves from the reticular framework.

#### PATHOLOGIC OBSERVATIONS ON THE RETICULUM OCCURRING IN THE SEVERAL TYPES OF GRANULOMA.

*Tuberculosis.*—In a tubercle the deposition of reticulum accompanies the proliferation and differentiation of reticuloendothelial cells; these become epithelioid cells. The fibers course in a rich network among these cells and also are seen among the peripherally situated lymphocytes. If complete caseation or liquefaction occurs, all details of the tissue are obliterated; reticulum is affected with the rest of the tissue and merits no special mention in this regard. It will, however, be observed frequently that tissue foci, varying greatly in color from yellow to white and in consistency from firm and fibrous to edematous or gelatinous, when sectioned and stained with hematoxylin and eosin will appear homogeneously pink and will show no detail, thus meriting a classification at least of coagulative necrosis; this completely characterless tissue nevertheless shows many reticular fibers when stained appropriately. The significance of this fact will be discussed after the findings in syphilis have been described.

*Syphilis.*—Reticulum is found in abundance in the gumma. It is present among proliferating plasma cells, lymphocytes and histiocytes, where no reticulum previously existed and where no fibroblast can be identified, and is seen in the walls of blood vessels. When the latter show a characteristic reduplication of the intima in layers, sections appropriately prepared show multiple concentric bands of reticulum. Since the necrosis in the gumma results from obliterative endarteritis with attendant ischemia, the tissue pattern remains to some extent, and reticulum is easily identified. It should be emphasized, however, that foci will be found in which all detail is lost.

Most authors comparing reticulum in tuberculosis with that in syphilis state that reticulum can be found in the gumma but rarely in the tubercle. Our finding is that although in the main the tendency for caseation to wipe out tissue structure is greater in tuberculosis, it is

unsafe to rely on the presence or the absence of reticulum as a crucial diagnostic factor. The pattern of the reticulum which is brought out in an apparently characterless tissue is of much greater significance than the amount. In the gumma the aforementioned concentric lamellas indicate the endothelial proliferation occurring in the original lesion. None of this is seen in the tubercle; in this structure, if anything different from the original tissue pattern is seen, it is of a type which would result from formation of fibers between semiradially disposed epithelioid cells. The difference thus is qualitative rather than quantitative.

*Blastomycosis.*—Miller<sup>25</sup> reported a case of pulmonary blastomycosis in which the reticular pattern did not differ from that in tuberculosis. In the single specimen available to us for study, a cerebral lesion, the granuloma contained a purulent element which dissipated the tubercular pattern and made it much less regular than that ordinarily seen in tuberculosis. In foci of polymorphonuclear infiltration reticulum is relatively scant. This results in a pattern of reticulum which is less regular than that in tuberculosis and in which blank pockets are found corresponding to microscopic abscesses. No specific feature is observed in the reticular pattern which is characteristic or of diagnostic significance.

*Coccidioidosis.*—The findings are essentially those of blastomycosis.

*Actinomycosis.*—The findings are the same as in the coccidioidal and the blastomycotic granuloma. In these three conditions, diagnosis must rest on finding the specific organisms. Incidentally, methods that demonstrate reticulum leave yeast and fungi strongly impregnated, and silver preparations in general, including strong protein silver, show *Actinomyces* as black filaments. Yeasts and fungi, however, can be shown as red bodies with carmine, according to the method of Kernohan,<sup>26</sup> even though strong protein silver is used to stain the background.

*Rheumatism.*—The reticular fibers in Aschoff bodies are found arranged irregularly in relation to the specific inflammatory cells. In the central fibrous cores of the rheumatic nodules the reticulum is seen intermingled with fibers which take the stain for collagen. Some broad fibrous masses give homogeneously the reaction for reticulum. Although this is an interesting observation, it is not of crucial diagnostic importance. The diagnosis of a rheumatic nodule must be made on the basis of features revealed by sections prepared routinely.

*Gout.*—In gout, round, ovoid or clover leaf foci are surrounded by encapsulating strands of reticulum and collagen in which atypical, characteristic aggregations of foreign body giant cells are found. Cleft for-

25. Miller, W. S.: Am. J. Path. 3:315, 1927.

26. Kernohan, J. W.: J. Neuropath. & Exper. Neurol. 2:95, 1943.

mation is prominent. In the central masses, homogeneous in routine sections except for clefts, very fine reticular fibers, almost disappearing into an amorphous state, are seen in sparse bundles which have an interweaving tendency and are related to the bundles of uric acid crystals and which, if they extend to the capsular bundles, merge with the latter. This picture is rather characteristic. It does not, however, add anything of great diagnostic value to the picture seen in sections stained with hematoxylin and eosin or those prepared with methods designed to show gout specifically.

*Sarcoid of Boeck*.—Reticulum is laid down by the epithelioid cells in a form similar to that noted in straightforward tuberculosis. Giant cells are seen infrequently and caseation scarcely at all, but there is no difference between the reticular patterns. This feature, therefore, shows a histologic affinity of the two conditions but is of no particular help in their diagnosis.

*Leprosy*.—The histiocytes of leprosy deposit reticulum freely. The pattern varies according to that of the histiocytes, as tuberculoid or lepromatous, and adds no information otherwise.

*Idiopathic Reticuloendothelioses*.—In Gaucher's disease, in the Niemann-Pick syndrome and in similar conditions, reticulum is deposited freely among the histiocytes. The amount varies; probably the more rapidly the reticular cells proliferate the less abundant is the reticulum. This appears to be true in a broad sense of inflammatory proliferations in general; for example, in lymphocytic infiltrations, when the cells pour forth rapidly, little or no reticulum may be found, whereas in a lymphocytic aggregation which gathers slowly and with some degree of organization, perhaps even simulating a follicle, reticulum may be abundant and even collagen may appear in varying quantities.

*Comment*.—In the specimens of granuloma available, certain specific characteristics are seen. While these are illuminating and are recorded for completeness, they are rarely of diagnostic value. As in the past, reticulum preparations are of value in distinguishing a gumma from a tubercle in certain cases. It is suggested that the presence or the absence of reticulum in such lesions be not depended on too strongly and that emphasis be placed on the obliterative endarteritic pattern of the gumma or the tuberculoid pattern in tuberculosis, wherever either of these can be demonstrated.

#### PATHOLOGIC OBSERVATIONS ON THE RETICULUM OCCURRING IN NEOPLASMS

*Tumors of Fibroblasts*.—Foot and Day<sup>14</sup> stated that fibroma contains abundant collagen but no reticulum. Mallory and Parker<sup>10</sup> were

of the opinion that tumors of fibroblasts contain reticulum but that the latter is only a form of collagen. We find that fibroma and fibrosarcoma contain abundant reticulum, and frequently collagen, with moderate variation. Collagen is less abundant in the cellular, anaplastic forms, but reticulum appears to be abundant in all forms, with no dependable relation to the degree of differentiation. Myxoma, myxosarcoma, or tumors of fibroblasts containing myxomatous elements have a property of forming reticulum similar to that of fibroma or fibrosarcoma. Neurofibroma, so-called, and fibroblastic tumors of the meninges will be mentioned later.

*Tumors of Muscle, Noncancerous and Cancerous.*—Myoma and myosarcoma were said by Foot and Day<sup>14</sup> to contain reticulum and collagen in varying degrees of abundance, reticulum perhaps being converted into collagen. We have been able to confirm this finding. Again, the degree of differentiation does not invariably predict the abundance of formation of reticulum. We also share the opinion of the same authors that rhabdomyosarcoma forms abundant reticulum. No reference was found to reticulum occurring in granular cell myoblastoma. Stout<sup>27</sup> expressed the opinion that in neoplasms of this type the amount of reticulum varies greatly. In the single specimen of granular cell myoblastoma available to us for study (incidentally, one behaving as a cancer), reticulum was found only investing the organoid nodules; it did not pass between the individual cells. This failure of an accumulation of muscle cells to conform to what appears to be a general rule concerning muscle and fibrous tissue is comparable to what has emerged as a general phenomenon in the course of this study: the unaccountable variability with which reticulum is deposited by cells of the same type in the same tissue section.

*Tumors of Fat Cells.*—Reticulum is deposited abundantly between the individual cells of lipoma and liposarcoma. In other words, adipose tissue acts in the same way as fibrous and muscular tissue.

*Tumors of Cartilage and Bone.*—Neoplastic tissue containing histologically benign cartilage and bone contains little or no reticulum. We agree with Foot and Day,<sup>14</sup> however, that cellular areas in chondrosarcoma or osteosarcoma show abundant reticulum and collagen. In this regard the chondroblast, the osteoblast, the fibroblast and the myoblast exhibit a familial relation.

*Foreign Body Giant Cell Tumors.*—Foot and Day<sup>14</sup> found these neoplasms not to be rich in reticulum and to contain only moderate amounts of collagen. It is our experience that reticulum and collagen

27. Stout, A. P.: Personal communication to the author.

are present in abundance, although with variation, especially in xanthomatous or granulomatous areas. These tumors thus compare with other connective tissue neoplasms or granulomas.

*Tumors of Blood and Lymph Vessels.*—As one would expect, hemangioma and lymphangioma show abundant reticulum outlining the walls of the vascular spaces, and collagen is present in adventitial tissue and areas of hyaline fibrosis. Hemangioendothelioma and lymphangioendothelioma (the picture in Lindau's disease will be described later) have reticulum outlining the blood spaces. Fibers radiate from the vessel walls and traverse the cellular tissue in abundance but vary in number from field to field and vary from a few fibrils tentatively exploring the periphery of the cell masses to a dense network investing nearly every cell. This illustrates again the variability of reticulum. It also shows that reticulum is found in the cell masses of malignant endothelioma. Foot and Day<sup>14</sup> stated that reticulum is found closely investing the cells of malignant endothelioma of lymph nodes. Our impression is that while, as already stated, reticulum is present and varies in amount, the degree of abundance on the average is moderate, not quite equaling the degree observed in sarcoma. A noteworthy exception to this whole group is Ewing's endothelial myeloma, considered widely to be a type of hemangioendothelioma. Foot and Day<sup>14</sup> found less reticulum and more delicate fibers in this than in the ordinary type of hemangioendothelioma. We have examined tissue from well documented specimens of Ewing's tumor and have found practically no reticulum except for that lining the blood vessels and a rare abortive perithelial twig. With regard to reticulum formation, Ewing's tumor differs not only from endothelioma but also from mesenchymal tumors in general. The failure to show reticulum in the cellular tissue of tumors of this type (Ewing's) is disappointing because it renders this staining method valueless in distinguishing Ewing's tumor from neuroblastoma—often a difficult distinction in the best hands and one leading to wide difference of opinion among eminent pathologists having abundant experience with these two tumor types, when they have reviewed sections belonging to one or the other of the two disease groups.

*Lymphosarcoma.*—Foot and Day<sup>14</sup> observed reticulum in lymphosarcoma, malignant reticuloendothelioma and Hodgkin's disease, and collagen in the latter, abundantly and without great discrimination. Callender<sup>28</sup> stated that in lymphatic leukemia, giant follicular hyperplasia and lymphosarcoma the reticulum of lymph nodes is not increased but is distended or separated by tumor cells. Foot and Day<sup>14</sup> found new reticulum deposited among lymphocytes where the tumor penetrated the capsule. We have found considerable variation in

28. Callender, G. R.: Am. J. Path. 10:443, 1934.

abundance of reticulum in lymphosarcoma and the nodes of patients with lymphatic leukemia but, on the whole, we have noted a generous amount, commonly investing individual cells. That new reticulum is formed is shown by its being deposited in extracapsular neoplastic tissue. One also should consider that if one were to reduce an average lymphomatous node to its original size, the reticular network would form a mat so dense that scarcely any room would be left for lymphocytes. We are in agreement with Foot and Day<sup>14</sup> that both reticulum and collagen are found in abundance in Hodgkin's disease, although the amount varies greatly. In sections of skin from a patient with mycosis fungoides, reticular and collagenous fibrils were present in abundance. The question of reticular cell sarcoma requires special attention. Cal-lender<sup>28</sup> stated that in reticular cell sarcoma a delicate network of reticulum is found investing every tumor cell, and he laid emphasis on this as a diagnostic feature. Foot and Day<sup>14</sup> in reference to malignant reticuloendothelioma reported finding some reticulum but added that it often traversed many cells without branching. Warren and Picena<sup>29</sup> stated that a dense network of reticulum might be observed, or only scattered, fragmented fine threads, little reticulum often being seen within the cell masses. They therefore minimized the importance of reticulum in the diagnosis of reticular cell sarcoma. This problem is complicated by the lack of general agreement as to just what reticular cell sarcoma is. Warren and Picena<sup>29</sup> used the term in a restricted sense, describing the reticular cells as the primitive mesenchymal cells and stating that in neoplastic formations they are seen in syncytial masses, the protoplasm being undivided or slightly fenestrated, with oval or indented nuclei, well defined nuclear membranes, sparse powdery chromatin granules and one or two prominent nucleoli to a cell, giving the nucleus an "empty" appearance. An admixture of lymphocytes was noted commonly, pure tumor types being found but infrequently, and the predominant cell type being used for classification. Ewing<sup>30</sup> showed one illustration which is obviously from a case of giant follicular hyperplasia, and various other contributors have differed widely in criteria of classification. We have come to regard the main cell of the germ follicle as a young lymphocyte. Actually, all of this makes little difference. We share the opinion of many others that lymphoid tumors overlap in cell type so extensively that they should be grouped together, a subclass being appended to the diagnosis in the making of a report, and type differences being used only as broad guiding principles in attempts to predict radiosensitivity and clinical course. We

29. Warren, S., and Picena, J. P.: Am. J. Path. 17:385, 1941.

30. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 425.

have observed further that reticulum is deposited in all forms of lymphosarcoma, including Hodgkin's disease, with wide variation and with no constant relation to cell type. In the reticular pattern of a histologic section of lymphosarcoma, nonfibrillar pockets may be found, with intervening foci of dense reticulum. Aside from natural variation, some of this may result from difference in rate of growth. In a certain case of lymphoblastoma cutis, in which one of numerous and repeated tumefactions appeared almost overnight, practically no reticulum could be shown. Reticulum is present indiscriminately in neoplastic and inflammatory nodes, and therefore is of no help in the diagnosis of borderline conditions. It is of interest that enlarging follicles of both adenitis and follicular lymphoma are relatively free of reticulum when compared with the surrounding lymphocytic tissue.

One must conclude, therefore, that reticulum is deposited indiscriminately by all members of the lymphoma family and is of little practical value in diagnosis; at least it adds nothing to the picture revealed by routine methods.

*Adenoma and Carcinoma.*—Epithelial neoplasms contain reticulum about the cell acini, but the acini themselves contain, if any, only rare, abortive peripheral reticular fibers. In this we are in accord with Foot and Day.<sup>11</sup> Reticular preparations may be used along broad lines in distinguishing epithelial from connective tissue or other mesenchymal neoplasms in difficult cases. It should be borne in mind, however, that occasionally carcinomatous acini can be quite small, and investment of these small acini may give a picture easily confused with that of sarcoma. This occurred in a pseudosarcomatous form of hypernephroma. In the group of epithelial neoplasms one may include seminoma, dysgerminoma and mixed tumors of the salivary glands (as regards epithelial acini). The relation between granulosa cell and theca cell tumor of the ovary deserves special comment. We have been able to confirm the finding of Wolfe and Neigus<sup>31</sup> that reticulum invests the individual theca cells but not the granulosa cells. Neoplasms of the anterior lobe of the pituitary gland behave as do adenoma and carcinoma of other glandular organs. No reticulum is found in the epithelial acini of lymphoepithelioma. We agree on this point with Cappell.<sup>32</sup> This tends to place lymphoepithelioma in the class of carcinoma, perhaps a modified squamous cell type.

*Tumors of Nerve Origin.*—We agree with Foot and Day<sup>14</sup> that glioma, ganglioneuroma, neuroblastoma and other tumors primarily of nerve origin contain no reticulum. Tumors of the meninges deserve special mention. We have been unable to recognize an endotheliomatous

31. Wolfe, S. A., and Neigus, I.: Am. J. Obst. & Gynec. 42:218, 1941.

32. Cappell, D. F.: J. Path. & Bact. 33:429, 1930.

form of meningioma in the sense commonly accepted. In 5 cases of true hemangioblastoma, in 1 of which the tumor was associated with Lindau's disease, reticulum was found outlining the blood vessels and extending into the cell acini in amount varying from scant to abundant. Usually the cell pattern shown by routine stains will reveal the angioblastic nature of these tumors. In 2 of our 5 cases, in both of which a diagnosis of astrocytoma had been made previously, it was possible to demonstrate both reticular and fine collagenous fibers radiating abundantly from the blood vessels among the tumor cells, and fat stains showed lipid droplets in the cytoplasm. Emphasis has been placed commonly on reticulum in hemangioblastoma in Lindau's disease, but study of most illustrations shows only the outline of the vascular spaces. This adds nothing to the hematoxylin-eosin or other routine preparation, which shows the characteristic picture of hypertrophied, often vacuolated endothelial cells forming acinous masses. Demonstration of reticular fibers among the individual cells, already mentioned, is entirely a different matter, and a positive result, if obtained, may be of great importance. It may be stated here that this principle is true of neoplasms in general. Reference is commonly found to the abundant reticulum found investing the acini of carcinoma and scattered in its stroma. It would be much more to the point to refer to the presence or the absence of reticulum among the individual tumor cells, and this should be the meaning in any unqualified reference to reticulum in a section of neoplastic tissue.

With regard to the more common varieties of meningioma (excluding hemangioma, which is like that elsewhere), namely, meningiotheliomatous, fibromatous (so-called dural endothelioma) and sarcomatous, we have found modest amounts of reticulum in the meningiotheliomatous type, occasional fibers penetrating the cell masses for varying distances, and abundant amounts in fibroma, fibrosarcoma and perithelial sarcoma, including perivascular lymphosarcomatous proliferations. In neuropathology this is the basis of a valuable and common use of methods of demonstrating reticulum. It can be stated with little reservation that every diffuse or cellular intracranial neoplasm not having some obvious specific feature requires preparation for the demonstration of reticulum and collagen along with the usual neurologic methods before one can say that it has been studied adequately. In this way, glioblastoma and sarcoma can be separated in many cases which otherwise might offer difficulty. Bailey,<sup>33</sup> and Globus<sup>34</sup> and practically every other discusser of intracranial sarcoma are agreed on this point.

33. Bailey, P.: Am. J. Clin. Path. **13**:478, 1943.

34. Globus, J. H.; Levin, S., and Sheps, J. G.: J. Neuropath. & Exper. Neurol. **3**:311, 1944.

Any tumor of a nerve sheath, which we regard as schwannoma in most cases, deposits reticulum abundantly and collagen irregularly. Cancerous schwannoma forms varying amounts of delicate reticular fibrils.

*Nevi and Melanoma.*—Benign and cancerous nevi contain some reticular fibers. Foot and Day<sup>14</sup> describe reticulum in melanosarcoma as penetrating the alveolar masses rather freely. Our own experience tends to place melanoma midway between sarcoma and carcinoma in content of reticulum. From this point of view, tumors of this type may be classed as modified epithelioma.

#### SUMMARY

Reticulum is a fibrillar substance closely related chemically and physically to collagen but differing from it on the basis of the staining by silver solutions, aniline blue, light green and other histologic reagents and of resistance to digestion by acids, alkali and gastric ferment. It is probably formed by intercellular precipitation of a secretion produced by reticular cells, histiocytes, monocytes, lymphocytes, vascular endothelium and fibroblasts, and also muscle, both smooth and striated. It is closely applied anatomically to the aforementioned cells but does not connect directly with the cell bodies. Reticulum is precollagen. Collagen is a chemical substance which impregnates fibers, probably reticular, thus forming what one knows as collagenous fibers.

Reticulum is demonstrated with ease and reliability sufficiently great to warrant use of appropriate methods in standard pathologic diagnosis. The method recommended embraces formaldehyde fixation, paraffin embedding, preparatory treatment of sections with potassium permanganate and oxalic acid, followed by immersion in silver nitrate solution, ammoniacal silver nitrate solution, solution of formaldehyde U. S. P., gold chloride solution, solution of oxalic acid and solution of sodium thiosulfate. Counterstaining, if used, consists of treatment with thionine or cresyl violet or with light green, ponceau and orange G.

Granuloma and lesions of the reticuloendothelial system produce reticulum in varying degrees of abundance, often in a characteristic pattern. The distinction of gumma from the tubercle of tuberculosis depends not so much on whether reticulum persists in necrotic tissue as on whether or not the lamellar endothelial reduplication in syphilis or the tuberculoid pattern in tuberculosis can be demonstrated; otherwise, the study yields nothing of diagnostic interest regarding the several types of granuloma. Methods that demonstrate reticulum render yeasts and fungi black.

Certain neoplasms are often distinguished with the help of methods for the demonstration of reticulum. Leiomyosarcoma, rhabdomyosar-

coma, fibrosarcoma, liposarcoma, the cellular portions of chondrosarcoma and osteosarcoma and other similar cancerous tumors of connective tissue origin, schwannian sarcoma and the theca cell tumor contain intercellular reticulum in varying degrees of abundance. Glioma, neuroblastoma, other tumors of purely neurogenic origin, adenoma, carcinoma, including seminoma, dysgerminoma, granulosa cell tumor, lymphoepithelioma, tumors of the anterior lobe of the pituitary gland, the epithelial portions of mixed tumors and Ewing's tumor contain no appreciable amount of intercellular reticulum. Melanoepithelioma contains an intermediate degree of reticulum. Hemangioendothelioma presents a picture in which the vessels are outlined, with reticulum penetrating to a varying degree into the cell masses, sometimes in great abundance. In this regard, endothelioma approaches sarcoma closely. Lymphosarcoma, leukemic adenosis, reticular cell sarcoma and Hodgkin's disease all present reticulum formation abundantly and indiscriminately, so that the presence of reticulum in this group will help to identify a tumor of mesenchymal origin but is of no help in subclassification. The study of any undifferentiated cellular neoplasm which is without obvious characteristics, whether located within the cranium or elsewhere in the body, is incomplete without preparation to show reticulum and collagen. The degree of abundance of reticulum varies among tumors of the same type and from field to field in the same section; the reticular pattern should therefore be employed as a histologic indication, although sometimes a strong one, among several in diagnosis rather than as a sole and conclusive criterion.

Much of the work on which this study is based was done in the laboratories of pathology of Western State Hospital, Fort Steilacoom, Wash., and the Clyde K. Emery Cancer Clinic, Los Angeles. The article was presented before the Indiana Association of Pathologists, Feb. 24, 1946.

## ROLE OF STASIS IN THE DEVELOPMENT OF PULMONARY INFARCTS

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THE role of the veins of the legs as a source of pulmonary emboli has been repeatedly emphasized.<sup>1</sup> It has been demonstrated experimentally that stasis is necessary if red cell thrombi are to be produced consistently by the intravascular insertion of foreign bodies.<sup>2</sup> While the importance of stasis in the development of thrombi in peripheral blood vessels is generally known, the role of stasis in the genesis of pulmonary infarction is not widely recognized. This is true despite the fact that Zahn<sup>3</sup> in 1897 applied the clinical observation that pulmonary infarction is more likely to occur if embolization occurs in the presence of passive congestion (stasis) by binding the thorax of the experimental animal (rabbits) two days after emboli had lodged in the lungs. In 1912 Karsner and Ash<sup>4</sup> in a comprehensive review of the literature and experimental study demonstrated the importance of stasis in pulmonary infarction and stated

. . . it can be seen clearly that simple embolism does not produce pulmonary infarction. . . . it may be safely said that not only is stasis a necessary corollary of the infarct, but also the greater the degree of stasis, the sooner is true infarction likely to appear.

The study reported here was undertaken to reinvestigate from the experimental standpoint the importance of stasis in the genesis of infarction following pulmonary embolism.

### EXPERIMENTS AND RESULTS

Rabbits anesthetized by intravenous administration of pentobarbital sodium were used. A pulmonary embolus was produced by inserting into the jugular vein a 2 cm. length of wool knitting yarn which had been previously soaked in

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1. Homans, J.: New England J. Med. **211**:993, 1934. Rossle, R.: Virchows Arch. f. path. Anat. **300**:180, 1937. Neumann, R.: ibid. **301**:708, 1938. Hunter, E. C.; Sneeden, V. D.; Robertson, T. D., and Snyder, G. A. C.: Arch. Int. Med. **68**:1, 1941. Homans, J.: New England J. Med. **231**:51, 1944.

2. Moses, C.: Federation Proc. **4**:52, 1945; Proc. Soc. Exper. Biol. & Med. **59**:25, 1945.

3. Zahn, F. W.: Verhandl. d. Gesellsch. deutsch. Naturf. u. Aerzte **69**:9, 1897.

4. Karsner, H. T., and Ash, J. E.: J. M. Research **27**:205, 1912.

defibrinated dog's blood and thoroughly dried. The yarn inserted into the vein was gently pushed with a thin glass rod into the right side of the heart, and the opening in the vein was repaired with fine silk.

*Controls.*—Eleven rabbits in which this procedure was carried out were killed at intervals of from one to seven days thereafter. In 8 of these animals bland pulmonary emboli were found without evidence of circulatory disturbance in the lung. In 3 animals the embolus had failed to pass beyond the right side of the heart and was found there.

*Compression of the Thorax.*—Five rabbits were used for this experiment. After the blood-impregnated yarn had been pushed into the right side of the heart, the chest was firmly compressed with three wide synthetic rubber bands ( $\frac{1}{2}$  by 3 in. [about 1 by 7.5 cm.]). In every one of these 5 animals hemorrhagic pulmonary infarction was evident in the lung containing the embolus within two to six days.

*Ligation of Pulmonary Vein.*—Ten rabbits sustained by intratracheal insufflation were used for this experiment. The thorax was opened, one of the pulmonary veins was ligated with linen thread, and the chest was quickly closed in an air-tight manner. None of these rabbits survived for more than sixteen hours, but hemorrhagic congestion involving the lobe drained by the ligated vein was evident in every animal within two to sixteen hours. Three of this group of animals were found at autopsy to have so-called "agonal thrombi" in the right side of the heart.

#### COMMENT

It is important to note that in all the experiments in which hemorrhagic pulmonary infarction was observed stasis and congestion were also present. In the rabbits without stasis bland embolism, without infarction, was noted. When, however, stasis and congestion were induced either by tying the pulmonary veins or by compressing the thorax, hemorrhagic infarction developed.

The tendency for "agonal thrombi" to develop in the right side of the heart was noted many years ago by Guthrie.<sup>5</sup> Boyd<sup>6</sup> commented on the frequency with which such thrombi are found in the right side of the heart of persons having pneumonia, which suggests the possible pathogenic role of stasis of the circulation in the right side of the heart in the presence of inflammatory pulmonary lesions.

From these observations it is evident that to prevent the development of pulmonary infarction, not only must stasis be prevented in the peripheral circulation to prevent the development of peripheral thrombi, but stasis and congestion in the pulmonary circuit must be avoided to prevent bland pulmonary embolism from yielding hemorrhagic infarcts. This does not mean that prevention of stasis in the pulmonary circulation can prevent fatal pulmonary embolism. Obviously, massive pulmonary embolism can mechanically prevent adequate oxygenation of the blood.

5. Guthrie, C. C.: *Blood Vessel Surgery*, London, E. Arnold, 1912.

6. Boyd, W.: *Pathology of Internal Diseases*, Philadelphia, Lea & Febiger, 1944.

## SUMMARY

Hemorrhagic pulmonary infarction could not be produced by the experimental method used in the absence of stasis and congestion in the pulmonary circulation. The observations emphasize the importance of preventing circulatory stasis not only in the systemic but also in the pulmonary circulation in order to prevent the development of hemorrhagic pulmonary infarcts.

# Case Reports

## INTESTINAL PERFORATION IN PARATYPHOID DUE TO SALMONELLA PARATYPHI B

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UP TO the present, approximately thirty cases of intestinal perforation in the course of paratyphoid have been cited.<sup>1</sup> In each of those cases the diagnosis of the type of *Salmonella* infection was based on the agglutination reactions of the patient's serum, on the biochemical and agglutinative properties of isolated organisms or on a combination of the two types of evidence; complete descriptions of bacteriologic investigations and analyses of antigenic structure were lacking. Because of the complete bacteriologic studies in the case to be reported, and the infrequency of pathologic reports of cases of this nature in American literature, it is thought worth while to publish our experiences.

### REPORT OF A CASE

The following clinical information was obtained from the records of a prison hospital. Feb. 16, 1944, a German soldier was admitted to the prison hospital for further treatment of a gunshot wound of the left shoulder, incurred five weeks previously. The shoulder wound was granulating and uninfected. The patient had a slight fever. Two days later, headache, vomiting and diarrhea developed. Examination revealed an elevated temperature and tenderness, rigidity and a palpable mass in the right lower quadrant of the abdomen. The leukocyte count was 15,600. A laparotomy on the evening of the same day revealed a normal appendix, an edematous lower part of the ileum and about 200 cc. of clear, yellow fluid in the abdominal cavity; the appendix was removed, and, on subsequent examination, was not remarkable. From February 18, the day of the operation, to March 3, the day of death, the patient had a fluctuating temperature, reaching a maximum of 39.8 C. (103.6 F.) on the first postoperative day and a normal level in the last two days. Two leukocyte counts were 5500 and 6500 respectively. Agglutination tests, one week after the operation, were negative for *Eberthella typhosa* and *Salmonella paratyphi* A and B; at this time a blood culture was positive for *S. paratyphi* B. A urinalysis showed albumin, urobilinogen and a diazo reaction. The patient was dyspneic and cyanotic during the last eight days. A blood pressure reading was 105 systolic and 80 diastolic; the pulse rate fluctuated between 120 and 140. Death occurred suddenly March 3, 1944. The

1. (a) Bonamy, P.: Les perforations intestinales au cours des paratyphoïdes: Différentes méthodes chirurgicales de traitement, Thesis, Paris, 1932. (b) Grenier: Paratyphoïde B avec perforation intestinale, Progrès méd. **29**:114, 1913. (c) Hohenschild, M.: Ulcus ilei perforatum nach Paratyphus, Thesis, Munich, Lübeck, Gebr. Hohenschild, 1927. (d) Papin, F.: Perforations intestinales au cours de paratyphoïdes, Bull. et mém. Soc. nat. de chir. **56**:1342, 1930. (e) Pop, A.: Ein Fall von Perforationsperitonitis bei ambulatem Paratyphus B, Zentralbl. f. Chir. **56**:77, 1929.

patient was given plasma and 50 per cent dextrose intravenously; a total dose of 13.5 Gm. of sulfanilamide was given between February 17 and 21.

*Autopsy* (fourteen hours after death).—The body was that of a well developed but emaciated young adult man. The left shoulder revealed a shallow ulcer, 13 mm. in diameter, with a red, granular base. The pleural cavities were free of fluid and adhesions; the pericardial cavity contained about 40 cc. of clear, yellow fluid. A deep cervical lymph node, the size of an almond, revealed uniformly tan parenchyma on sectioning. About 300 cc. of thick yellow pus was in the abdominal cavity; the intestinal loops were coated irregularly by sheets of yellow fibrin and bound to each other by easily broken, fibrinous adhesions; the appendix was absent. The mesenteric lymph nodes were slightly enlarged, and, on section, presented uniformly pink parenchyma. The small intestine was distended, and parallel loops lay transversely in ladder pattern.

The esophagus, the stomach, the duodenum and the jejunum revealed no mucosal abnormalities. The mucosa of the lower third of the ileum displayed shallow ulcers on the antimesenteric side; the majority of the ulcers were oval, and their long axes were parallel to the long axis of the intestine. The bases of the ulcers varied; some were rough and brown with tan peripheries; others were streaked finely with gray and spotted with red. There were several widened, elongated and slightly depressed Peyer's patches, covered over with rugated mucosa. Two ulcers presented small perforations; one was 4 cm. and the other was 8 cm. from the ileocecal valve; the serosa around the perforations was covered with thick, easily removable yellow sheets of fibrin. The mucosa of the proximal half of the ascending colon presented small round superficial ulcers, averaging 5 mm. in diameter and less than 1 mm. in depth; their bases were smooth and tan, and their margins were occasionally elevated. The rest of the colon revealed no mucosal abnormalities.

The left lung weighed 352 Gm.; the right, 625 Gm.; there was uniformly diminished crepitance in all lobes; sections revealed deep red parenchyma oozing considerable serosanguinous fluid; the trachea and the bronchi contained foamy pink fluid. The heart weighed 280 Gm.; there were no abnormalities other than a flabby myocardium. The liver weighed 2,060 Gm., was increased in size and normal in shape; the capsule was flecked with strands of fibrin; the parenchyma was reddish brown finely mottled with tan. The spleen weighed 150 Gm. and was normal in size and shape; the capsule was partially covered with sheets of fibrin; the organ sectioned with ease, exposing reddish purple pulp, from which a moderate amount of diffuent material was removed on scraping. The gallbladder and the pancreas were not remarkable. The adrenal glands were larger and softer than normal; sections displayed light yellow cortex, accentuated vascular markings in the zona reticularis and gray medulla. Each kidney weighed 147 Gm.; the capsules were stripped with slight difficulty, rarely pulling away small pieces of cortex; the cortical surfaces were otherwise smooth; sections revealed grayish red parenchyma with accentuated cortical vascular striations; the pelves showed scattered mucosal petechiae. The ureters, the urinary bladder, the prostate and the testes were not remarkable. The brain weighed 1,358 Gm.; the only abnormalities revealed were accentuation of the vascular markings of the white matter throughout and a cyst, 3 mm. in diameter, in the midportion of the pituitary gland; the cyst contained gray, opaque, viscid material.

*Microscopic Examination*.—The pathologic diagnosis was: acute passive congestion of the lungs; atheroma of the aorta; focal necrosis and fatty degeneration of the liver; acute passive congestion of the spleen; subacute interstitial pancreatitis; parenchymatous degeneration, edema and focal necrosis of adrenal glands;

perforation of the ileum, hyperplasia and ulceration of Peyer's patches and paratyphoid fever; ulceration and colitis of the ascending colon, acute fibrinous peritonitis; focal necrosis and acute lymphadenitis of mesenteric and cervical lymph nodes; acute passive congestion and parenchymatous degeneration of the kidneys; cyst of Rathke's pouch, acute passive congestion of the brain.

In the lung, numerous alveolar spaces and bronchiolar lumens were filled with structureless eosinophilic granular material and scattered erythrocytes. There was dilatation of the capillaries of the pleura, the alveolar septums and the bronchiolar mucosa. Rare clumps of streptococci and plump rods were found in the alveolar spaces. There were no other abnormalities.

The heart was not remarkable. The aorta contained one focal intimal collection of lipoid cells. In the liver, several central and midzonal areas revealed loss of lobular structure; enmeshed in a fine fibrillar eosinophilic network were disjointed and shrunken parenchymal cells, erythrocytes and rare round cells and granulocytes. Elsewhere the basic lobular structure was preserved; the parenchymal cells revealed swelling, haziness of cell outline and occasionally numerous small intracytoplasmic vacuoles; the vacuolated cells had no particular lobular localization. The liver cords were often broken; the central veins and sinusoids were moderately dilated. Scattered Kupffer cells contained intracytoplasmic erythrocytes. The periportal spaces were not abnormal.

The capsule of the spleen contained scattered neutrophils. The trabeculae and the malpighian follicles were not remarkable. The venous sinuses of the red pulp were dilated; erythrocytes occupied the sinuses and the interstitium. Occasional endothelial cells contained intracytoplasmic erythrocytes. In one small area in the red pulp the sinus structure was almost completely obliterated by erythrocytes; enmeshed in the red cells were occasional endothelial cells, granulocytes and pyknotic nuclear debris, and at the periphery were numerous endothelial cells and scattered granulocytes.

In the adrenal gland, the cortical cells were separated from one another, and the capillaries were dilated. The cells of the zona fasciculata often lacked vacuolation, were swollen and had dense granular cytoplasm; scattered cells were enlarged and had almost completely vacuolated cytoplasm and hazy outline. Numerous cells of all zones were shrunken, with pyknotic or pale nuclei. A rare monocyte was present within the cords. In the zona fasciculata and reticularis there was one small focus of obliteration of cord pattern; the parenchymal cells were shrunken, vacuolated and pale, and their nuclei were pyknotic, fragmented, pale or absent; numerous round cells and neutrophils and eosinophilic granules were present in the focus.

Acinous cells of the pancreas occasionally revealed swelling and vacuolated pale cytoplasm. Acini and intralobular ducts were often distended with eosinophilic granular material; a rare neutrophil was enclosed. The intralobular and perilobular interstitium contained scattered round cells and rare granulocytes. A hemolymph node in the peripancreatic adipose tissue revealed in its sinuses numerous monocytes with intracytoplasmic erythrocytes.

Section near a site of perforation of the ileum revealed that centrally the structural pattern of all layers was completely replaced by structureless granular and fibrillar eosinophilic material with enmeshed nuclear debris and scattered clumps of bacteria. At the intact margins the mucosal pattern was largely obscured by numerous round cells, granulocytes, fine eosinophilic fibrils and coarse eosinophilic granules; small islands of lymphoid tissue were also present. The submucosal and muscular layers were infiltrated with round cells, chiefly monocytes; the submucosal vessels were dilated. The serosa was largely replaced by branching and anastomosing bands of fibrin with enmeshed neutrophils and nuclear debris.

Section through an enlarged Peyer's patch displayed hyperplasia of the lymphoid tissue of the mucosa; in some areas the lymphoid tissue was shallow and infiltrated with numerous monocytes; the glandular portion of the mucosa was largely absent over the lymphoid tissue, and revealed extensive postmortem degeneration elsewhere. The submucosa, and to a lesser extent the muscular layer, revealed numerous monocytes and scattered lymphocytes and granulocytes in the interstitium. The serosa was acutely inflamed as in the section through the area of perforation. The colon revealed focal absence of the mucosa and several small collections of round cells and granulocytes enmeshed in granular and fibrillar eosinophilic material at the base of the defect, in the submucosa.

The perinodal adipose tissue and capsules of mesenteric lymph nodes were focally infiltrated with monocytes, lymphocytes and occasional neutrophils. Sinuses were widened and filled with leukocytes, chiefly monocytes; the latter often contained intracytoplasmic vacuoles, nuclear debris or erythrocytes. Parenchymal capillaries were dilated, and scattered monocytes, plasma cells and granulocytes were in the interstitium. Germinal centers were absent. An arteriole was filled with fibrin and leukocytes. Both sinuses and parenchyma revealed scattered patches of granular and fibrillar eosinophilic material with round cells, nuclear debris, granulocytes and anucleate cells with pale pink cytoplasm. A cervical node revealed similar inflammatory changes to a lesser degree; in addition, the parenchyma contained discrete patches of dense fibrous tissue, in which were scattered extravascular clumps of erythrocytes, granulocytes, round cells and capillaries.

Glomerular loops and peritubular capillaries of the kidney were dilated; collecting tubules contained scattered hyaline and granular casts and crystals. The crystals were opaque, oval or round, and occasionally radially striated; some were irregular in shape. Cells of convoluted tubules were slightly swollen and hazy in outline; the lumens contained granular eosinophilic material. There was one small collection of lymphocytes and monocytes in the interstitium of the medulla. The pelvis was not remarkable.

Sections of cerebrum, cerebellum, pons and basal ganglia revealed similar changes. Meningeal and parenchymal capillaries were dilated. Rare Virchow-Robin spaces contained erythrocytes. Occasional neurons were shrunken, and their nuclei were pale. The glial tissue was not remarkable. The pars intermedia of the pituitary gland contained one large round space and numerous small round or irregular spaces lined by ciliated cuboidal epithelium and filled with structureless eosinophilic bars, granules and fine fibrils. The lining cells had granular eosinophilic cytoplasm and round or oval vesicular nuclei. Rare smaller spaces were lined by low cuboidal, nonciliated epithelium. The large space was surrounded by a thin band of dense fibrous tissue. The anterior and the posterior lobe of the pituitary gland were not abnormal.

At autopsy, samples were taken of heart blood, spinal fluid, Peyer's patch, spleen, mesenteric lymph node, peritoneal fluid, bile, urine and feces. The material was streaked directly on desoxycholate-citrate plates and placed in selenite F enrichment medium; the latter, after eighteen hours' incubation, was streaked on the plating medium. *S. paratyphi B* (*Salmonella schottmüllerii*) was identified from all specimens, several colonies being picked from each plate. Typing of the culture was done by the method of Edwards and Bruner.<sup>2</sup> The

2. Edwards, P. R., and Bruner, D. W.: Serological Identification of *Salmonella* Cultures, Circular 54, Kentucky Agricultural Experiment Station, 1942.

antigenic formula was IV, V, XII, . . . : b-1, 2. The identity of the organism was confirmed by Captain D. W. Bruner, Veterinary Corps, A. U. S. The biochemical reactions were typical. The organism produced hydrogen sulfide and utilized sodium citrate; it did not act on sodium *d*-tartrate, litmus milk or gelatin and did not form indole. Dextrose, rhamnose, xylose, dulcitol, mannitol and maltose were fermented, with production of acid and gas; lactose, sucrose and salicin were not fermented.

#### COMMENT

Among interesting findings at autopsy were two perforations of the ileum and a spleen of normal size and weight. Multiple perforations have been seen previously,<sup>1a</sup> and splenic size varies considerably,<sup>1c</sup> in paratyphoid. Pathogenic organisms were cultured from the spinal fluid, but the brain revealed no inflammatory response. A cervical lymph node displayed inflammatory changes similar to those seen in the mesenteric lymph nodes.

#### SUMMARY

In a case of perforation of the ileum in paratyphoid the outstanding clinical features were fever, headache, vomiting, diarrhea, abdominal pain, dyspnea and cyanosis. The early symptoms and signs suggested appendicitis and led to appendectomy. The major findings at necropsy were acute fibrinous peritonitis, two perforations of the ileum, hyperplasia and ulceration of Peyer's patches, ulceration of the ascending colon, acute lymphadenitis of mesenteric and cervical nodes and focal necrosis of lymph nodes, of the liver and of the adrenal glands. *S. paratyphi B* (*S. Schottmüller*) was isolated at autopsy from various organs and body fluids.

AN ACUTE FEBRILE ILLNESS CHARACTERIZED BY THROMBOPENIC  
PURPURA, HEMOLYTIC ANEMIA AND GENERALIZED  
PLATELET THROMBOSIS

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HERE is a small but growing group of cases of an acute febrile illness characterized by marked and rapidly progressing anemia and thrombopenia. The first reported case was described by Moschcowitz<sup>1</sup> in 1925. Since then, to our knowledge, 7 additional cases have been reported.<sup>2</sup> These have all been similar clinically in that the patients had definite thrombopenia, rapidly progressing anemia and a febrile course terminating in death in from nine days to three weeks. In all the reported cases the patients have been females. Death has always been preceded by rather severe but nonlocalizing cerebral signs.

Pathologically, too, the reported cases have been similar in that petechial hemorrhages and platelet thrombi were present throughout the body.

We report here a case in which the clinical and pathologic aspects are similar to those described previously. Some new pathologic features are described, but nothing is added to the knowledge of the genesis of the disease. This is the first account of the appearance of this syndrome in a male.

REPORT OF A CASE

A 24 year old Italian baker was admitted to the Milford Hospital with rather severe vomiting of a few hours' duration. Two weeks previously he had had a severe cold with fever, prostration and cough but no sore throat. Convalescence was slow, and malaise continued. Three days before admission he felt generally ill and noticed hematuria. At that time laboratory studies revealed the following data: hemoglobin 11.5 Gm. per hundred cubic centimeters, red blood cells 3,500,000

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From the Mallory Institute of Pathology, Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Milford Hospital, Milford, Mass.

1. Moschcowitz, E.: Arch. Int. Med. **36**:89, 1925.
2. (a) Baehr, G.; Klempner, P., and Schifrin, A.: Tr. A. Am. Physicians **51**:43, 1936. (b) Friedberg, C. K., and Gross, L.: Arch. Int. Med. **58**:641, 1936. (c) Gitlow, S., and Goldmark, C.: Ann. Int. Med. **13**:1046, 1939. (d) Altschule, M. D.: New England J. Med. **227**:477, 1942. (e) Bernheim, A. I.: J. Mt. Sinai Hosp. **10**:287, 1943.

per cubic millimeter, white blood cells 8,700 per cubic millimeter. No platelets were seen in a stained film of peripheral blood. There were a few purpuric spots on the anterior abdominal wall. At 2 a. m. on the morning of admission he began to vomit.

His past history revealed no familial anemia or purpura of any type. There had been no exposure to or ingestion of any toxic drug, with particular reference to benzene, organic solvents in general, the sulfonamide compounds or fava beans.

In the hospital he was in a state of semiconsciousness, restless and uncooperative. His temperature was 101 F., and the blood pressure was 120 systolic and 70 diastolic. The pulse was rapid. He was pale and slightly jaundiced, with petechiae over the skin of the abdomen and that of the lower legs, in the mouth and in the conjunctivas. There were large petechiae and scratch marks on the face, over which there had been itching. The chest was clear with symmetric expansion. The heart was not enlarged, and no cardiac murmurs were heard. The abdomen was flat and not tender. There were a few palpable, nontender, freely movable small cervical lymph nodes, with no other superficial evidence of lymphadenopathy.

The laboratory data at this time were as follows: hemoglobin 36 per cent, hematocrit reading 18.5 per cent, red blood cells 2,180,000 per cubic millimeter, white blood cells 9,200 per cubic millimeter, icteric index 20. A differential count of the white blood cells revealed polymorphonuclear leukocytes 37 per cent, band forms 17 per cent, small lymphocytes 23 per cent, adult monocytes 17 per cent, eosinophils 4 per cent, basophils 2 per cent and blast forms 1 per cent. There were two nucleated red blood cells per hundred white blood cells. Reticulocytes composed 10 per cent of the red blood cells. The stained film of peripheral blood revealed moderate polychromatophilia of the red blood cells and marked thrombopenia. The osmotic fragility test gave the following data: hemolysis of 1 per cent of red blood cells in a saline solution with a concentration of sodium chloride of 0.51 per cent, 10 per cent hemolysis in 0.40 per cent saline solution, 50 per cent hemolysis in 0.33 per cent saline solution and 75 per cent hemolysis in 0.26 per cent saline solution. Cold agglutinins could not be demonstrated.

The patient remained comatose and became more jaundiced. Several transfusions of compatible blood failed to alter the progress of the illness. He became more irritable until his condition was that of restless stupor. He was entirely disoriented and unaware of his surroundings. On the third hospital day he died, approximately fifteen days after the onset of his present illness.

The clinical diagnosis was acute hemolytic anemia and acute thrombopenia, type and cause unknown.

*Autopsy* (six hours after death).—The body was that of a well developed and well nourished young white man, 175 cm. long and weighing approximately 145 pounds (65.5 Kg.). The skin was slightly icteric. Small petechiae were scattered over the body, the conjunctivas and the buccal membranes. The forehead bore many large petechiae and superficial lacerations consistent with scratch marks.

The smoothly lined abdominal cavity contained about 200 cc. of clear yellow-brown fluid. The peritoneal surfaces showed no petechiae. The pleural cavities were dry, showed no petechiae and were smooth except for an area of old fibrous adhesions at the right apex. Both the parietal and the visceral pericardium were covered by many petechiae.

The heart weighed 370 Gm., being slightly heavier than normal for the patient's size. The endocardial surface of the right auricle and ventricle, like the pericardium, contained many petechial hemorrhages. The endocardium of the left auricle and ventricle showed no petechiae.

The right lung weighed 580 Gm. and the left 630 Gm. The cut surface revealed many areas of hemorrhage, most marked in the lower lobes. The spleen was moderately enlarged and weighed 370 Gm. Except for its size it appeared grossly normal. The liver weighed 1,915 Gm., being moderately enlarged, with a smooth, yellow to brown mottled surface. The kidneys were congested and weighed 435 Gm. There were several small petechiae in the renal pelvis. The adrenal glands were of normal size and weighed a total of 17 Gm. Many hemorrhages, 1 to 2 mm. in diameter, were scattered along the periphery of the cortex. The vertebral and the sternal marrow were deep red. The marrow in the midfemoral region was yellow, with a thin layer of red marrow immediately subjacent to the bony cortex. The lymph nodes in the abdomen and the mediastinum were more prominent than usual, being 0.5 to 0.8 cm. in diameter. On section their cut surface was gray and wet. The brain weighed 1,420 Gm. The gyri were flattened, indicating moderate cerebral edema. There were no visible hemorrhages or areas of softening.

*Bacteriologic Report.*—On culture of the heart's blood no pathogenic organisms grew out. Both lungs contained moderate numbers of hemolytic *Staphylococcus aureus*.

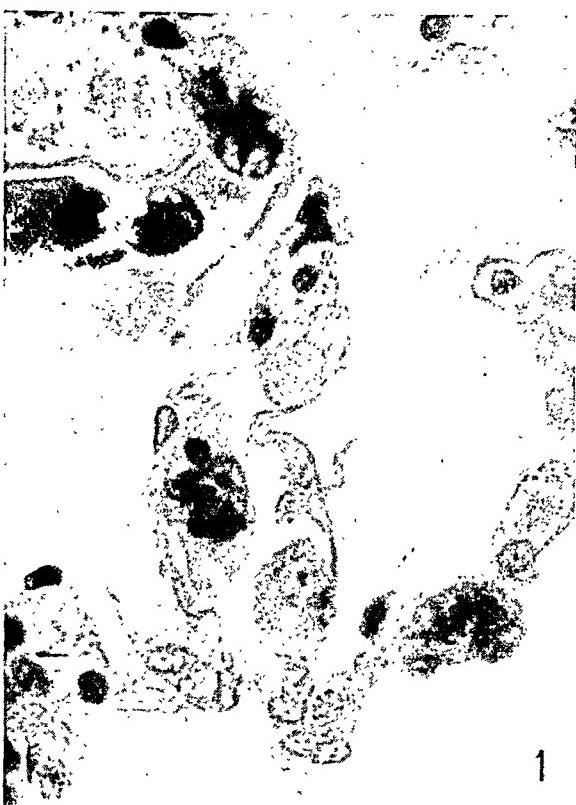
*Microscopic Examination of the Organs.*—**Heart:** There were many areas of hemorrhage throughout the myocardium of the right ventricle and throughout the left ventricle except for a subendocardial zone 2 mm. deep. In the areas of hemorrhage many occluded and partially occluded capillaries were found. The occlusions were produced by thrombi composed of finely granular material staining light pink with the phloxine and methylene blue stain. With the exception of two small thrombi along the walls of small arteries in the myocardium, all the lesions appeared in capillaries and larger, thin-walled vessels consistent with small veins or the transition vessels between capillaries and veins. Many capillaries with no thrombi showed definite swellings of endothelial cells. Mitotic figures were found in some of these endothelial cells. A few lesions showed some perivascular infiltration with large mononuclear leukocytes and occasional lymphocytes. A more complete description of these lesions will be given in a later section. Rare areas of myocardial necrosis were found associated with these thrombosed vessels.

**Lungs:** The lungs showed many small areas of hemorrhage and a few foci of bronchopneumonia. The capillaries contained only a rare, questionable thrombus. A great number of megakaryocytes were in the small vessels throughout both lungs (fig. 1). Some of these cells were so large that they extended into three adjacent microscopic sections, each section being 9 microns thick.

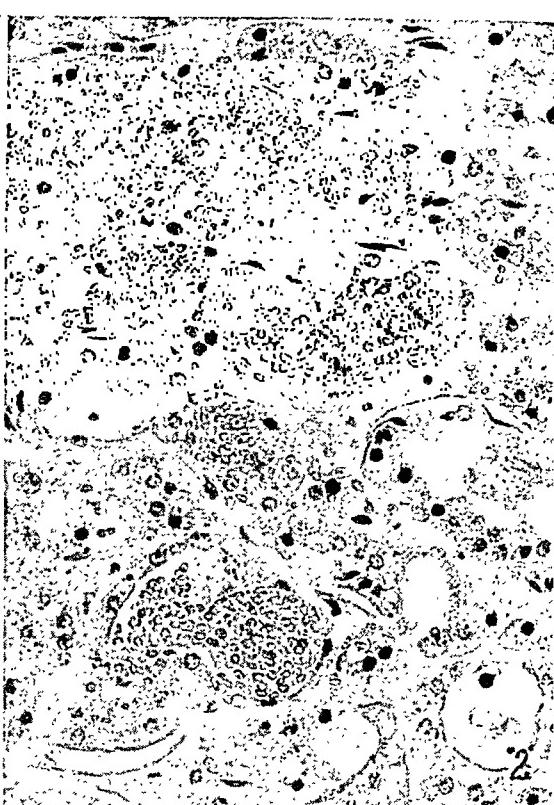
**Spleen:** The spleen was markedly congested. The malpighian corpuscles were well outlined, smaller than usual and inactive. No capillary thrombi were present, and a few megakaryocytes were seen. Occasional erythroblasts were present, but no significant amount of myeloid metaplasia was noted.

**Liver:** Many of the capillaries in the portal areas were thrombosed, but no such lesions were present in the sinusoids or large vessels. There was fatty metamorphosis of the hepatic parenchyma, and a few lymphocytes and an occasional polymorphonuclear leukocyte were found in the portal areas. The Kupffer cells contained a moderate amount of yellow-brown pigment and some phagocytosed red blood cells.

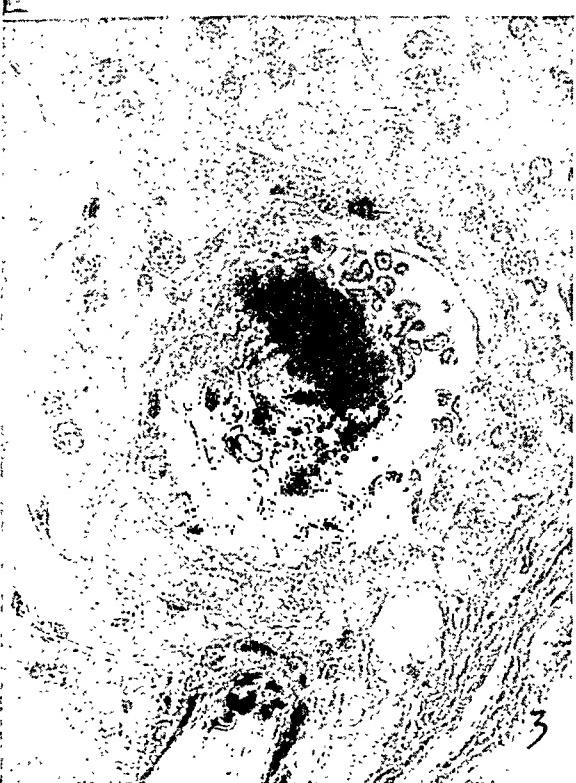
**Kidneys:** Many of the capillaries and small veins in the deeper layers of the cortex and in the medulla near the cortex contained thrombi similar to those present elsewhere in the body. Many of the glomerular tufts presented these lesions, but only rare thrombi could be found in the arterioles connected with the tuft. Several convoluted tubules were filled with fresh red blood cells, but no hemoglobin casts



1



2



3

*(See legend on opposite page)*

could be demonstrated. There was necrosis of some cells of the convoluted tubules, with regeneration, as evidenced by the presence of mitotic figures. Scattered tubular cells contained droplets characteristic of colloid droplet degeneration.

**Adrenal Gland:** Sections of adrenal gland showed marked lumen formation in the zona glomerulosa and zona fasciculata. Many hemorrhagic areas were present in the peripheral half of the cortex. The hemorrhages were in the lumens just mentioned and appeared to be recent and of the same age (fig. 2). Platelet thrombi of varied age were present in the capillaries and small arterioles and the veins of the zona glomerulosa. The endothelial cells lining these vessels showed definite swelling, proliferation and occasional mitotic figures. Migrating leukocytes were associated with many of the lesions. These leukocytes were found in the thrombi, at their periphery and lying free in the adjacent lumens (fig. 3). Small areas of necrosis were found in the periphery of the cortex.

**Vertebral and Sternal Marrows:** There was rather marked hyperactive erythropoiesis, which was predominantly at the erythroblastic level. Megakaryocytes appeared in the usual numbers, but the majority contained dark, pyknotic nuclei consistent with the appearance of old megakaryocytes. Many of the capillaries contained thrombi.

**Brain:** Sections of brain showed a few capillary thrombi with no hemorrhages.

**Other Organs:** Vascular lesions (thrombi) were also present in lymph nodes, the pancreas and the intestine.

**Microscopic Examination of the Thrombi.**—In order to substantiate the work of others and to determine as clearly as possible the nature of the capillary occlusions, several differential stains were used on tissues fixed in Zenker's solution or in solution of formaldehyde U. S. P. (1:10).

For routine examination tissues were stained with phloxine and methylene blue after fixation in Zenker's solution. Other stains<sup>3</sup> used were hematoxylin and eosin, Mallory's aniline blue connective tissue stain, Weigert's fibrin stain, phosphotungstic acid-hematoxylin, Giemsa stain (Wolbach's modification), Van Gieson and elastic tissue stains and iron stains. Sato's method was used for peroxidase granules.

Thrombi of varied appearance were found. We classified them as early, intermediate or old. We considered the earliest stage to be a thrombus composed of finely granular material which contained no red blood cells, fibrin, leukocytes, hyalin, collagen or bacteria. In some instances the early type of thrombi completely

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3. Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938.

#### EXPLANATION OF FIGURE 1

Fig. 1.—Section of lung showing megakaryocytes filling capillaries. Phloxine and methylene blue;  $\times 700$ .

Fig. 2.—Periphery of the cortex of an adrenal gland illustrating marked lumen formation and fresh bleeding into these lumens. Phloxine and methylene blue;  $\times 260$ .

Fig. 3.—A vessel in the cortex of an adrenal gland with an old dense endothelialized thrombus surrounded by monocytes and polymorphonuclear leukocytes. Van Gieson and elastic tissue stains;  $\times 400$ .

Fig. 4.—Two vessels in the cortex of an adrenal gland illustrating the three stages of lesions described in the text. The larger vessel contains a thrombus of intermediate age overlaid with freshly precipitated platelets. The smaller one is partially filled by an old dense thrombus. Van Gieson and elastic tissue stains;  $\times 400$ .

&gt;

occluded the capillary lumen, while in others it was apparently deposited along one part of the wall. The accumulated granular material became more compact and fused to form the transition or intermediate type of thrombus (figs. 4 and 5). The third type was made up of a dense, homogeneous material closely adherent to the vascular wall, and in most instances was covered by endothelial cells. In our opinion thrombi of this type were old thrombi. Endothelial cells lining vascular channels had proliferated and covered the thrombi. Various combinations of these three types or stages were found. These combination forms were definitely laminated, and in some of them endothelial cells separated the different layers (figs. 4 and 6).

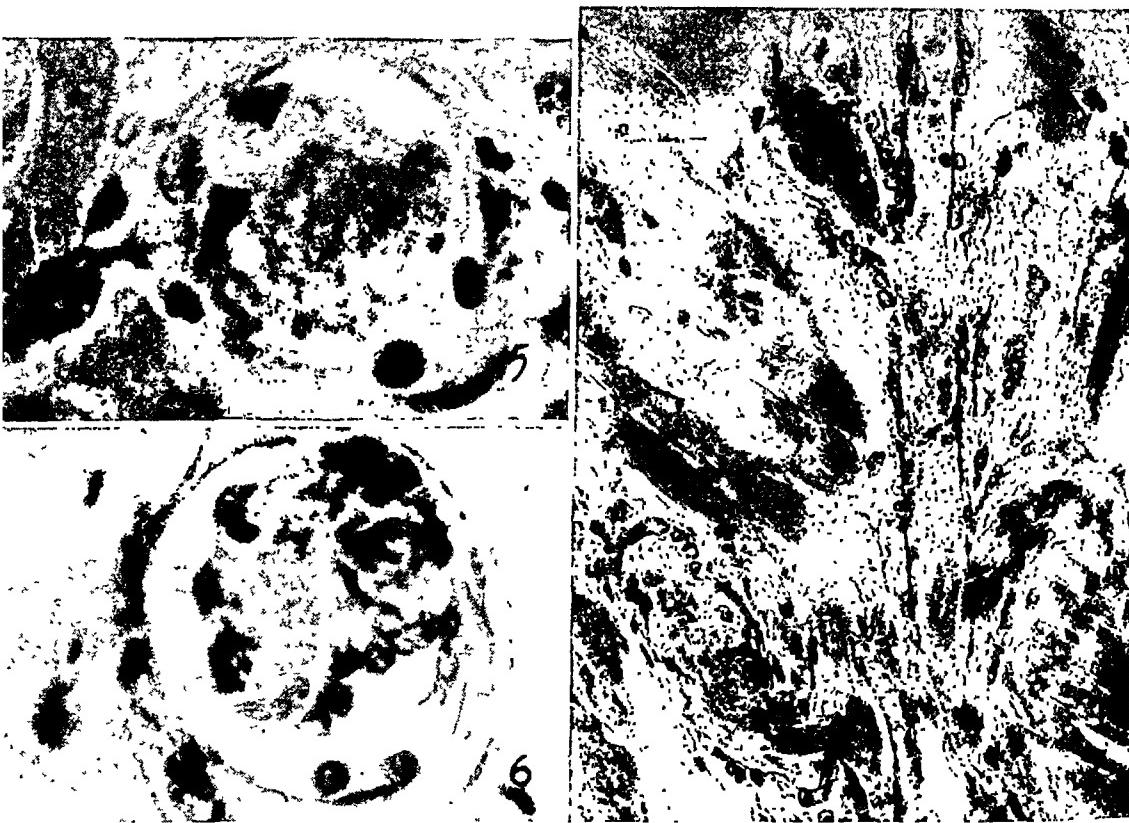


Fig. 5.—A small vessel with swollen endothelial cells contains a thrombus made up of early fused platelets and freshly precipitating platelets. Giemsa stain;  $\times 725$ .

Fig. 6.—A laminated thrombus, covered by proliferated endothelial cells, almost completely occluding a small myocardial vessel. Giemsa stain;  $\times 725$ .

Fig. 7.—The various stages of the developing platelet thrombi are demonstrated in this branching myocardial vessel. The lumen of the main channel, lined by swollen endothelial cells, is filled with the fresh granular material. The branches show older, dense thrombi. Van Gieson and elastic tissue stains;  $\times 275$ .

Most of the lesions occurred in capillaries, some in small veins and a few in arterioles. A capillary was identified as a small, endothelium-lined lumen with no muscularis or elastic membrane. A venule or small vein was defined as a small, thin-walled vessel containing an occasional muscle fiber and having a very thin, incomplete elastic membrane. A small vessel with a well developed muscularis and a prominent elastic membrane was considered as an arteriole.

With the aniline blue stain, the finely granular substance was colored bluish pink, the old, homogeneous material a deep, dense red, and sometimes the intermediate, or early fused, layer took a deep blue. The Van Gieson method showed no pink-staining material consistent with collagen in any thrombus. The phosphotungstic acid-hematoxylin stain failed to demonstrate a fibrillar substance in any of the lesions. There was no fibrin and no iron-containing pigment. From their staining reactions and morphologic aspects these thrombi were considered to be made up primarily of platelets.

The lesions of dense material filled with nuclei resembled organized thrombi. However, stains for collagen failed to demonstrate its presence in any lesion. In a few of the lesions showing layers of different ages, the intermediate stage of the thrombus stained a homogeneous blue with the Mallory aniline blue method. The older, final stage never took this blue color, but always a deep red. No thrombus showed any suggestion of a pink stain with the Van Gieson method. In view of the aforementioned observations and the fact that the aniline blue stain may color many different materials a deep blue, we conclude that none of these lesions showed any organization or fibroblastic activity.

In the affected areas almost every capillary contained prominent endothelial cells with vesicular nuclei (fig. 7). These were present in uninvolved as well as in involved capillaries, but were more noticeable in the capillaries showing thrombi. The proliferation of, and mitotic figures in, these endothelial cells have already been mentioned. The migrating leukocytes in and around the lesions were peroxidase positive, consistent with monocytes.

#### COMMENT

From the findings we agree with Altschule<sup>2d</sup> and with Baehr, Klemperer and Schifrin<sup>2a</sup> and the others that these lesions are platelet thrombi deposited on vessel walls. Altschule suggested that the thrombi may have resulted from deposition of platelets on damaged endothelium. The swollen endothelial cells which we observed rather diffusely throughout the involved organs may represent the earliest histologic evidence of such endothelial damage. Following the deposition of the platelets, there is an increase in the density and compactness of the thrombus. Endothelial cells swell, proliferate and cover the platelet thrombus (fig. 6). On this endothelialized surface more platelets can deposit and again become covered by endothelial cells. The process forms a lesion which contains nuclei and appears organized but in which there are neither fibroblasts nor collagen.

The clinical picture of this illness with its rapid febrile course, thrombopenia, severe anemia and death preceded by severe cerebral signs is characteristic of the other cases, previously described. The decreased erythrocyte count, the lowered hemoglobin level, the increased icteric index and clinical jaundice, the 10 per cent reticulocyte count, the moderate hemosiderosis of the spleen and the liver and the hyperplastic marrow satisfy our criteria for hemolytic anemia. The anemia developed to a marked degree prior to the appearance of the bleeding tendency. Histologically the small hemorrhages appeared to be quite recent, no hemolysis of red blood cells or deposition of iron-containing pigment being shown in these areas. These facts indicate that the jaundice was due to an intravascular hemolytic process and not to extravasation and breakdown of erythrocytes in the tissues.

We believe that the pathologic observations in this case do not suggest that the condition is in any way related to Werlhof's idiopathic disease known as purpura hemorrhagica, periarteritis nodosa, lupus erythematosus disseminata or other diseases of the erythema group. The histologic appearances are similar to those observed in animals in which the Shwartzman phenomenon had been produced.<sup>4</sup>

#### SUMMARY

A fatal febrile illness occurring in a young man was associated with rapidly developing hemolytic anemia and thrombopenia. Postmortem examination revealed many small hemorrhages and widespread platelet thrombosis of the small blood vessels. The type and the distribution of these vascular lesions are pathognomonic of illness of this type.

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4. Shwartzman, G.; Klempner, P., and Gerber, I. E.: J. A. M. A. **107**:1946, 1936.

## EPIDERMOID CARCINOMA ARISING IN AN ENDOMETRIAL CYST OF THE OVARY

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and  
HENRY C. FALK, M.D., NEW YORK

PRIMARY epidermoid carcinoma of the ovary is rare. Its usual source is the stratified squamous epithelial lining of a cystic teratoma (dermoid cyst), which Ewing<sup>1</sup> mentioned. One of us (H. C. F.)<sup>2</sup> has encountered 3 cases in which the carcinoma was of that origin. In the present case it seems to have had another origin, the epithelial lining of an endometrial cyst of the Sampson<sup>3</sup> type. In this instance it is analogous to the epidermoid carcinoma of the uterine fundus but shows no mixture of adenomatous elements as in adenoacanthoma. It shows no evidence of teratomatous origin, no sebaceous glands, cartilage, nerve tissue or other teratoid element. The carcinoma appears confined to the wall of the cyst, though locally invasive. No evidence of metastasis is apparent.

To diagnose such a carcinomatous transformation of an ordinary ovarian cyst preoperatively is almost impossible. In the present case the carcinoma represents only an incidental finding in material routinely examined.

### REPORT OF A CASE

An unmarried white woman, 61 years old, was admitted to St. Johns Riverside Hospital, Yonkers, N. Y., July 13, 1944, complaining that her abdomen had slowly and steadily increased in size for the past five years and that there had been increasing constipation for one and one-half years and lately pain in the abdomen. Her father died of cancer at 65 years; the mother, of an accident.

Her health was always good. Ten years before, she had hives lasting ten days, the result of ingestion of seafood. Six years before, she had a growth on the right side of the upper lip, which was treated by roentgen ray, with no recurrence or visible metastasis. Menstruation ceased eight years before, with no bleeding since. For six days she had noted pain in the right upper abdominal quadrant, rather severe, and slight shortness of breath in the miderect position. A mass could be felt in the lower part of the abdomen, apparently ovarian and cystic, large and without tenderness. Her general appearance indicated good nourishment. The pupils were equal and reacted to light and distance. There

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From the Surgical Service and Laboratory, St. John's Riverside Hospital, Yonkers, N. Y.

1. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 679.

2. Falk, H. C.: Personal communication to the authors.

3. Sampson, J. A.: Arch. Surg. 3:245, 1921.

was an old scar on the right side of the upper lip. The cervical nodes were not enlarged. The thyroid gland was not enlarged. No dyspnea was noted; the breath sounds were clear, with no pulmonary dulness; there was slight impairment of respiratory excursion. The heart sounds were of good quality, without



Fig. 1.—Lining of simple, low columnar epithelial cells at the mouth of a wide crypt. Lymphoid stroma is seen on one side.  $\times 608.5$ .

Fig. 2.—Narrow tubular gland.  $\times 608.5$ .

Fig. 3.—Lining of transitional epithelium with flattened surface cells.  $\times 608.5$ .

Fig. 4.—Epidermoid carcinoma showing one mitotic figure and two keratotic whorls.  $\times 608.5$ .

murmurs; there was no apparent hypertrophy. The abdomen showed no evidence of free fluid. The liver was not palpable. The extremities showed no tremor and no edema; the reflexes were normal. The blood pressure was 148 systolic and 80 diastolic.

The blood count on admission showed hemoglobin 10.7 Gm. per hundred cubic centimeters, erythrocytes 4,620,000 per cubic millimeter, color index 0.86 and leukocytes 13,300 per cubic millimeter; the differential count was basophils 1 per cent, eosinophils 4 per cent, staff neutrophils 5 per cent, segmented neutrophils 63 per cent and lymphocytes 22 per cent. There were no red cells of abnormal morphologic aspect. The urine varied in specific gravity from 1.006 to 1.016 and showed no albumin, sugar or acetone, few leukocytes and no blood or casts. The total protein of the blood was 6.83 Gm. per hundred cubic centimeters, the serum albumin 3.9 Gm. and the globulin 2.9 Gm.; the albumin-globulin ratio was 1.34. The blood type was Landsteiner O, Rh negative.

Operation, July 15, 1944, revealed a large cyst of the right ovary, unilocular, containing an estimated 4,000 cc. of thick, clear, dark brown fluid. The pedicle was attached to the right infundibulopelvic ligament, and there was a band of tissue adhering to the posterior peritoneal wall. The cyst and the attached right fallopian tube were removed.

The cyst showed a tough wall with many slight rough elevations of its lining, which otherwise was smooth. Histologic examination showed occasional areas which resembled endometrium, with few shallow glandular crypts, some dilated, lined by low columnar epithelial cells, which covered some of the nearby surface. Beneath this epithelium, small areas resembling endometrial stroma occurred, with densely packed small stellate cells and a few groups of large histiocytes. The thickened areas of the lining showed stratified squamous epithelium in apparent neoplasia. There were loss of polarity of the squamous cells, variation in size and shape, keratotic whorls, variation in size and pigmentation of nuclei and many mitotic figures. Bordering the thick epithelial masses were thinner sheets of stratified epithelium of the squamous and transitional types, sometimes undermining the columnar epithelium adjacent, showing loss of polarity of the cells. The wall of the cyst was formed of dense fibrous tissue, invaded by many small groups and masses of the neoplastic squamous cells. Considerable inflammatory reaction was present, especially about areas of tumor.

The postoperative course was uneventful for a short time. After three months, constipation and pain in the abdomen returned, followed by urinary frequency, pus and blood in the urine. Examination revealed a mass the size of a lemon in the left broad ligament. The patient was hospitalized in another institution, and death followed signs of uremia, Jan. 12, 1945. Permission for an autopsy was not obtained.

To determine whether vitamin A deficiency was related in any way to the changes in the lining of the cyst, a record of the diet was obtained for the week after discharge. This was representative of the diet followed for many years. It showed no deficiency, yielding about 8,000 U. S. P. units of the vitamin daily, occasionally over 10,000. This bears out the finding that the squamous metaplasia of vitamin A deficiency occurs in non-neoplastic epithelium only, while epidermoid carcinoma commonly occurs in regions where epithelium other than stratified squamous is found, such as the bronchus, the urinary tract or the endometrium, in persons who show no evidence of this deficiency.

#### SUMMARY

Epidermoid carcinoma was observed arising in the epithelium of an endometrial cyst of an ovary.

## Obituaries

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### JOSEPH McFARLAND, M.D.

1868-1945

Joseph McFarland was born in Philadelphia, Feb. 9, 1868, several months after the death of his father, who succumbed to pulmonary tuberculosis. He early became interested in science. With his mother, he frequently visited museums of natural history. When 15 years old he lectured at the Lauderbach Academy, now extinct, on the story of a pebble.

McFarland graduated from the medical school of the University of Pennsylvania in 1889. An inspiring teacher was William Osler, who advised him to spend ten years studying pathology as an introduction to medicine. In pursuance of this, the young man went to Heidelberg, Germany, where he studied under Julius Arnold and worked in bacteriology under Paul Ernst. Later he went to Vienna, Austria, and took morbid anatomy under Kolisko and neuropathology under Oberheimer. Several years later he made the acquaintance of Rudolph Virchow and worked for a time in Fränkel's laboratory at Halle, Germany.

His first appointment was at the University of Pennsylvania, that of assistant to the professor of pathology under John Guiteras at a salary of \$50 a year. Six months later he was made demonstrator in pathology at \$1,000 a year. On this income he married Virginia Kinsey in 1892, and he "lived with her for fifty years without a single quarrel."

In 1894 he took on the professorship of pathology in the Philadelphia Polyclinic Hospital and College for Graduates in Medicine, holding it for two years without salary and without a single student. He then resigned to accept the lectureship on bacteriology, the first at the University of Pennsylvania, and began to prepare for his first book, "The Pathogenic Bacteria." He was at this time and for the next six years director of the new laboratory for biologic products opened by the H. K. Mulford Company, who began the manufacture of antidiphtheritic serum. When the laboratory outgrew its quarters and moved to Glenolden, Pa., and a full time director became imperative, he withdrew. Almost immediately Parke, Davis & Company offered him the post of consultant, which he held for ten years and resigned to become a full time professor.

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This account is based on information given by Dr. Helen McF. Woodbridge, Dr. McFarland's daughter, and on his autobiographic sketch.



Joseph McFarland (2)

For the next twenty years, McFarland held the chair of professor of bacteriology and pathology in the Medico-Chirurgical College, until its amalgamation with the University of Pennsylvania. He then became professor of pathology with Allen J. Smith, when "began . . . the most happy period of my institutional life, for Smith and I were thoroughly congenial and our association perfectly harmonious." He lectured on general pathology in the veterinary and dental schools of the university, holding also the rank of professor of pathology in the Woman's Medical College of Pennsylvania, where he succeeded A. O. J. Kelly and was followed by Fred D. Weidman when forced to resign to give full time to the university. On his retirement for age, he at once became professor of pathology in the Temple University Dental School, a post which he held at the time of his sudden death, Sept. 22, 1945. He was pathologist to several hospitals and served the Philadelphia General Hospital (Blockley) as such for more than thirty years.

He was a member of the College of Physicians of Philadelphia, the American Medical Association (secretary and chairman of the section on Pathology and Bacteriology), the American Association of Pathologists and Bacteriologists (a founder), the Philadelphia Pathological Society (president), the American Society of Clinical Pathologists (honorary member) and other associations.

Dr. McFarland was a versatile scholar and prolific writer. He read easily German, French and Spanish. He published some 200 medical papers, contributed special chapters to systems and textbooks, and wrote the following books: "Text Book upon Pathogenic Bacteria" (nine editions), "Text Book of Pathology," "Biology—General and Medical," "The Breast" (with John B. Deaver), "Fighting Foes Too Small to Be Seen" and "Surgical Pathology." He made important contributions to the study of tumors of the salivary glands.

He was the minister for several years in a struggling church, preached frequently in the summer places to which he went, and made many commencement addresses. He was an excellent lecturer, enjoyed a keen sense of humor, and was a friendly and helpful guide to young physicians.

In World War I McFarland was commissioned a major in the Medical Corps of the United States Army in April 1917 and served as chief of the laboratory services at various hospitals in this country. He acquired pulmonary tuberculosis in the latter part of 1919, was given hospital treatment, and was discharged in February 1920.

In 1942 the Philadelphia Medical Society conferred the Strittmater Award on McFarland as being the outstanding medical man in Philadelphia in 1941.

I. DAVIDSOHN.

## Notes and News

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**Appointments, Etc.**—Lieutenant Colonel G. Howard Gowen has been appointed chief of the division of cancer control in the Illinois Department of Public Health.

Elmer L. Sevringshaus, professor of medicine at the University of Wisconsin, has accepted an appointment as director of clinical research for Hoffmann-La Roche, Inc.

Harry Goldblatt has resigned as professor of experimental pathology and associate director of the institute of pathology at Western Reserve University School of Medicine, Cleveland, effective in August, to become director of an institute for medical research at Cedars of Lebanon Hospital, Los Angeles.

**Grant for Study of Tropical Diseases.**—The Marcelle Fleischmann Foundation has made a grant of \$20,000 a year for a ten-year period to the department of public health and preventive medicine of Cornell University Medical College for the study of tropical diseases. The specified purpose of the gift is to promote the study of immunologic and allergic manifestations of exotic diseases and to provide for a better understanding of their prevention and treatment in relation to other allergic diseases. The investigations at Cornell will be under the direction of Morton C. Kahn.

**Award.**—The American Foundation for Tropical Medicine has made the third award of the Richard Pearson Strong Medal for outstanding achievement in the field of tropical medicine to Brigadier General George R. Callender, United States Army, director of the Army Medical School.

**American Board of Pathology.**—This board will hold examinations in San Francisco, July 1 and 2, if there are a sufficient number of applicants. Pathologists interested should submit their applications at once to Dr. F. W. Hartman, secretary, Henry Ford Hospital, Detroit 2, Mich.

**The Army Institute of Pathology.**—The Meritorious Service Plaque was awarded on Feb. 7, 1946 to the Army Institute of Pathology. The letter of transmittal from the Surgeon General to the director of the institute, Colonel J. E. Ash, reads in part as follows: "Under the provisions of Circular 345, War Department 1944, as amended by Section 1, Circular 331, 1945, I take great pleasure in awarding the Meritorious Service Plaque to the Army Institute of Pathology, your command, for superior performance of an exceptionally difficult mission and for untiring devotion to duty of the personnel of this unit during the period July 1945 to 31 December 1945."

## Books Received

A. A. A. S. RESEARCH CONFERENCE ON CANCER: A CONFERENCE OF PAPERS AND DISCUSSIONS PRESENTED AT THE SUMMER MEETING OF THE SECTION ON CHEMISTRY OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE AT GIBSON ISLAND, MARYLAND, JULY 31-AUGUST 4, 1944. Publication Committee: Dean Burk, chairman; Ralph G. Meader, John J. Bittner and Vincent du Vigneaud. Edited by Forest Ray Moulton. Pp. 333, illustrated. Washington, D. C.: American Association for the Advancement of Science, 1945.

This volume represents well the status in 1944 of research on certain fundamental problems of cancer. There are five sections with well prepared papers and pertinent discussions on: the virus approach to the etiology of cancer, carcinogenesis, enzymes, diet, experimental chemotherapy. The conference and the report are of great credit to the American Association for the Advancement of Science as well as to the participants. The understanding of cancer has been advanced.

PREVENTIVE MEDICINE AND PUBLIC HEALTH. By Wilson G. Smillie, M.D., D.P.H., Sc.D. (hon.), professor of public health and preventive medicine at Cornell University Medical College, New York. Price \$60. Pp. 607. New York: The Macmillan Company, 1946.

The special feature of this book is its point of view that the teaching of preventive medicine, that is the protection and the promotion of personal and family health, should be done as an essential part of the teaching of clinical medicine. "The content of the text brings out the fact that teaching of preventive medicine will be most effective if it is integrated with the teaching of clinical medicine. Thus the members of the departmental staff in preventive medicine should have clinical appointments, and should teach their subjects, not from a textbook, but at the bedside, in the clinic, and in the home." The book is a highly important contribution to advance the teaching of preventive medicine.

JOURNAL OF THE HISTORY OF MEDICINE AND ALLIED SCIENCES. Volume 1, number 1, January 1946. Pp. 183. Price \$7.50 a year in the United States, Canada and Latin America; \$7.80 elsewhere. Published quarterly. New York: Henry Schuman, 1946.

This quarterly will have no rival in this country except the *Bulletin of the History of Medicine*, edited by H. E. Sigerist, which largely reflects the work of the Baltimore school. Competition, however, is disavowed; George Rosen, the editor of the new journal, says: "it will not compete with, but will supplement the *Bulletin*." Associated with Rosen to constitute the board of editors, are Erwin R. Ackerknecht, Max H. Fisch, John F. Fulton and Josiah C. Trent. The international scope of the publication is shown by an impressive list of 43 consulting editors of North and South America and many countries in Europe. The format is pleasing. In the 183 pages of this first number are eleven scholarly articles that are well worth reading. A "Notes and Queries" section under the charge of Max H. Fisch may well prove to be one of the most attractive features of the magazine. All who are interested in medical history will wish the new journal a full measure of success.

PROTOZOLOGY. By Richard R. Kudo, D.Sc., professor of zoology at the University of Illinois, Urbana. Third edition. Pp. 778, with 336 illustrations. Price \$8. Springfield, Ill.: Charles C Thomas, Publisher, 1946.

TABLES OF REPRESENTATIVE VALUES OF FOODS COMMONLY USED IN TROPICAL COUNTRIES. By B. S. Platt. Medical Research Council, Special Report Series, no. 253. Pp. 41. Price 25 cents. London: His Majesty's Stationery Office, 1945.

**BOVINE TRICHOMONIASIS.** By Banner Bill Morgan, Ph.D., parasitologist and assistant professor of veterinary science, University of Wisconsin, Madison. Pp. 150, illustrated. Price, \$3.25. Minneapolis, Minn.: Burgess Publishing Company, 1945.

Bovine trichomoniasis is a venereal disease and an important cause of abortion and sterility in cows. It is curious that from 1897 to 1925 *Brucella abortus* was regarded as the only cause of infectious bovine abortion. It seems as if somehow *Trichomonas foetus*, easily seen under low magnification, was overlooked in smears of exudate examined under high power. Bovine trichomoniasis has been found to be widely distributed and to cause serious losses in animal husbandry. Prevention requires that infected bulls be not used for breeding. Morgan's monograph is a comprehensive summary of the literature on the disease, to the knowledge of which he himself has made significant contributions. The bibliography contains 408 items, almost all published since 1925. The description of *T. foetus* is complete in all respects. In other parts the monograph is more a rather rambling catalogue of facts than a compact, closely organized composition. The arrangement, into chapters is rather loose. The last chapter, headed "Miscellaneous," contains statements that should have been incorporated into other chapters. There is no index. But there can be no question about the value of the monograph as a reliable source of information of all kinds about bovine trichomoniasis.

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## Book Reviews

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- A **Study of Endometriosis, Endosalpingiosis, Endocervicosis, and Peritoneo-ovarian Sclerosis. A Clinical and Pathologic Study.** By James Robert Goodall, M.D., formerly professor of clinical gynecology and obstetrics at McGill University. Second Edition. Pp. 151, with 19 illustrations. Price \$5.50. Philadelphia: J. B. Lippincott Company, 1944.

In the preface the author states that in his further research he "has found only corroborative evidence to substantiate the facts and opinions expressed in the first edition." The only major change appears to be the adding of a section on endometriosis of the urinary system (pages 77 to 88) to the chapter on extrauterine endometriosis. Criticisms, factual and otherwise, in reviews of the first edition remain unnoticed. The book is an elaborate and repetitious exposition of the author's observations and ideas on endometriosis, but there is no thorough review and analysis of the literature on this topic. The first chapters deal briefly with matters of historical, classificational and nomenclatural interest. Uterine endometriosis is divided into intrauterine and parietal, each composed of glands and stroma, that is "mixed," or of stroma only. The relation of the intrauterine or "endometrial endometriosis" to endometrial hyperplasia as currently understood is not clearly defined. Then come more detailed chapters on uterine endometriosis, the stromal form of which, it is claimed, does not respond in any way to the cyclic influences that affect the endometrial glands. The forms and the sites of extrauterine endometriosis are described in chapter 11. The rest of the book takes up the causation, the clinical manifestations and the treatment of endometriosis and its effects, also allied conditions, e. g., peritoneal and ovarian sclerosis. The original illustrations are inadequate. There is no clean-cut, convincing illustration of stromal endometriosis. Its relations to fibroma, myoma and sarcoma, though much discussed, remain vague. While the book contains much instructive information about forms, locations and results of endometriosis, the frequently repeated explanations of its growth are speculative. It is not known, as claimed on page 13, that the action of estrogen is limited to the outer two thirds of the endometrium. It is not conclusively settled that endometriosis originates

only in the endometrium. That endometriosis is an expression of "vitiated endocrine function" or of excess in estrogen production is not definitely known. In the paragraphs on "endocervicosis" also assumption takes the upper hand. The following dogmatic statement (page 15) appears to overlook reports of cases of endometriosis in pregnancy: "Were all the endometrial cells susceptible to the menstrual hormones, the destructiveness of endometriosis would be increased tremendously, or were it possible for active endometriosis and pregnancy to co-exist, the destructiveness of endometriosis would be inconceivably increased, owing to the great surcease of the ovarian hormones during pregnancy. Fortunately for womankind active endometriosis and pregnancy *never* co-exist." (The italics occur in the original.)

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## PATHOLOGIC ASPECTS OF ACUTE EPIDEMIC HEPATITIS, WITH ESPECIAL REFERENCE TO EARLY STAGES

Report of a Series of Ten Cases, Including a Case in Which There Was Spontaneous Rupture of the Spleen and Six Cases of Fulminating Disease in Patients Who Had Been Wounded Several Months Previously

COMMANDER DAVID A. WOOD, MC(S), U.S.N.R.\*

**E**PIDEMIC hepatitis is a disease which has long been of importance in military medicine and which, as a result, receives the greatest attention in time of war. There is considerable confusion in regard to the etiologic agents and the nomenclature of the disease. It has been and is known by a variety of names—"epidemic catarrhal jaundice," "campaign jaundice," "acute catarrhal epidemic hepatitis," "infectious hepatitis," "acute infective jaundice" and "infective jaundice." "Homologous serum jaundice" is a term applied to a type of jaundice which develops occasionally as a sequel to the transfusion of plasma or whole blood or to the inoculation of biologic products containing human serum. The etiologic agents in epidemic hepatitis and homologous serum jaundice appear to be quite similar and may even be identical. None of the various synonyms just cited is especially satisfactory. Current usage here and abroad favors the term "epidemic hepatitis." Even though epidemic hepatitis and homologous serum jaundice may be caused by the same agent or by agents which differ only slightly antigenically, it may be well for the time being to continue the use of the two terms, to emphasize not only the difference in route of infection but differences in period of incubation and in severity of clinical course.

In peacetime, as a rule, only isolated cases are encountered, although periodically there have occurred scattered epidemics among civilians. The isolated or sporadic cases have been referred to as endemic

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\* On military leave of absence from the Department of Pathology, Stanford University School of Medicine.

hepatitis. Most authorities are of the opinion that endemic and epidemic hepatitis are one and the same disease, although the matter is still controversial. Conditions occasioned by campaigns, and possibly transfusions of whole blood or plasma containing the icterogenic agent in the field, give rise to factors which are favorable for outbreaks of the disease.

Recent experiments have shown definitely that the disease is due to a filter-passing agent, probably a virus. Havens,<sup>1</sup> also Neefe and co-workers,<sup>2</sup> as well as others,<sup>3</sup> have recently produced hepatitis in human volunteers by a number of methods. Their experiments emphasize three important features: (a) The "virus" is in the feces; (b) the disease can be produced by feeding either serum, feces or nasal washings to human subjects; (c) inoculation of comparable material usually gives rise to a longer period of incubation than does feeding.

The striking difference in incubation period, depending on whether the virus is inoculated or enters by way of the alimentary tract, is one of the unexplained peculiarities of the disease. According to Paul,<sup>4</sup> in the former case the period of incubation varies from fourteen to one hundred and sixty days, with a general range of sixty to one hundred and twenty days, in contrast to a period of from twenty to forty days in the latter case. In the latter instance the incubation period conforms more closely to that observed clinically. There are occasional cases, however, in which there is no history of known contact but in which a transfusion of blood or plasma has been given two to three months previously. Various studies of the 1942 outbreak in the Army of the United States following the use of yellow fever vaccine suggest clearly that the hepatitis (homologous serum jaundice) which has been produced in such cases was due not to the yellow fever virus in the vaccine but to the virus of epidemic hepatitis. At that time human serum was used in preparation of yellow fever vaccine.<sup>5</sup> Observations made following administration of icterogenic material to human volunteers conformed closely to clinical observations. For example, Neefe and co-workers<sup>2</sup> showed that a much longer period of incubation ensued when the icterogenic material was intro-

1. Havens, W. P., Jr.: Experimental Production of Infectious Hepatitis by Feeding Icteric Materials, in Conference on Liver Injury, Sept. 18-19, 1944, New York, Josiah Macy, Jr. Foundation, 1944, pp. 78-80.

2. Neefe, J. R.; Stokes, J., Jr.; Rienhold, J. G., and Lukens, F. D. W.: J. Clin. Investigation 23:836, 1944.

3. Findlay, G. M., and Martin, N. M.: Lancet 1:678, 1943.

4. Paul, J. R.: Hepatitis: Clinical and Epidemiological Aspects, in Conference on Liver Injury, Sept. 18-19, 1944, Josiah Macy, Jr. Foundation, 1944, pp. 75-77.

5. Turner, R. H.; Snavely, J. R.; Grosman, E. B.; Buchanan, R. N., and Foster, S. O.: Ann. Int. Med. 20:193, 1944.

duced intravenously than when it was introduced into the alimentary tract. Quite uniformly in clinical cases of homologous serum jaundice following yellow fever vaccination long incubation periods have been recorded similar to those observed in human volunteers. In the army outbreak of 1942 it was observed that the interval between the date of yellow fever vaccination and the onset of the disease varied from forty to one hundred and twenty days, with the onset occurring as much as six months later in a few cases. In the Navy, DeVeer and Matzner<sup>6</sup> reported a fatal case of yellow fever vaccination in which the onset of symptoms occurred four months after the transfusion of blood or plasma. Beeson<sup>7</sup> reported 7 cases of jaundice occurring one to four months after transfusion of blood or plasma. Intervals of seventy-eight to eighty-three days were noted by Propert<sup>8</sup> in 7 children who had received inoculums from the same batch of measles convalescent serum. Three of the children died and showed the lesions of "acute yellow atrophy." Findlay and Martin<sup>9</sup> transmitted the disease to human volunteers by transferring nasal washings from patients with jaundice due to fever vaccine. They accomplished the transmission by intradermal instillation and noted incubation periods of twenty-eight, thirty and fifty days. Voegt<sup>10</sup> observed jaundice four weeks after oral ingestion of duodenal juice. The incubation period for acute epidemic hepatitis is variously stated, but the following figures are representative: twenty to forty days (Paul<sup>11</sup>), twenty to thirty-one days (Newman<sup>10</sup>), twenty-six to thirty-five days (Pickles<sup>11</sup>) and fourteen to thirty-seven days (Ford<sup>12</sup>).

The manner in which epidemic hepatitis spreads is unknown. Neefe<sup>2</sup> in the light of experiments already cited is of the opinion that it is spread by droplet infection directly, by fomites indirectly. In a study of a recent civilian epidemic at a boys' and girls' camp in Pennsylvania Neefe and Stokes<sup>13</sup> demonstrated evidence which indicated that the icterogenic agent was water borne, the epidemic being secondary to fecal contamination of well water. In this study they failed to demonstrate the presence of the causative agent in either the nasal washings or the urine of 26 and 38 patients, respectively. In epidemics, several modes of transmission may be concerned. Of epidemiologic importance is the peculiar seasonal incidence—a sharp rise in the autumn months and an equally sharp drop in winter.

6. DeVeer, J. A., and Matzner, M. J.: U. S. Nav. M. Bull. **42**:1381, 1944.
7. Beeson, P. B.: J. A. M. A. **121**:1332, 1943.
8. Propert, S. A.: Brit. M. J. **2**:677, 1938.
9. Voegt, H.: München. med. Wchnschr. **89**:76, 1942.
10. Newman, J. L.: Brit. M. J. **1**:61, 1942.
11. Pickles, W. N.: Brit. M. J. **1**:944, 1930.
12. Ford, J. C.: Lancet **1**:675, 1943.
13. Neefe, J. R., and Stokes, J.: J. A. M. A. **128**:1063, 1945.

Fortunately, the disease is usually mild, with a low mortality rate. In the different localities and different armies in which epidemics have occurred, this rate has varied from 0.13 to 0.44 per cent.<sup>14a</sup> As a result of the low mortality, there has not been until recently much opportunity to study thoroughly the morphologic aspects of the disease.

From the standpoint of morphologic anatomy, knowledge of epidemic hepatitis has been rather meager, especially that pertaining to cases in which the disease is fatal in the early stages and to those cases in which acute "homologous serum jaundice" occurring in previously wounded men is fatal a few days after the onset of jaundice. Inasmuch as most of the data referable to the morphologic anatomy of this disease have been obtained from cases in which the infection has run a protracted course, little is known about the incipient and early changes, some of which may later disappear or subsequently become secondarily altered or completely obscured. It is well recognized that the principal lesions are found in the liver, although associated lesions have been described—principally those of the spleen and the regional lymph nodes. Descriptions of hepatic, splenic and lymph node lesions have almost uniformly been limited to those cases in which the disease ran a course varying from ten days to four months, with an average of one to two months. In 8 of the 10 cases to be reported in this paper, the clinical course varied from two to ten days. As will be discussed later, it is suspected that in at least 5 and probably 6 of these cases the icterogenic agent was introduced in plasma or whole blood transfusions, and that these cases might well be classified as instances of "homologous serum jaundice." In these cases not only characteristic lesions were seen but other early lesions, hitherto undescribed. Some of the latter were probably transient, as the presence of such lesions had not been previously noted or emphasized in the more protracted clinical cases.

Essentially the hepatic lesion as recognized at the present time is one of irregular distribution which involves the hepatic cells (hepatocellular necrosis) without damaging the sinusoids or the reticular framework. Destruction (autolysis) of the damaged liver cells is thought to occur rapidly. Because of the irregular involvement, large areas of parenchyma may be destroyed while elsewhere there is no destruction or it is incomplete. Limitation of the destructive process to the liver cells with preservation of lobular outlines (due to non-destruction of sinusoids and reticular framework) is thought to be highly characteristic of epidemic hepatitis. In the earlier phases the stroma is variably infiltrated by inflammatory cells (lymphocytes, mononuclear phagocytes and leukocytes in varying proportions). Even so,

14. Lucke, B.: (a) Am. J. Path. 20:471 and (b) 595, 1944.

no scarring occurs—merely condensation of preexistent stroma. The destructive process initially involves those liver cells situated about the central lobular venules. Later it may progress so as to involve either part or all of the lobule. Frequently the central lobular venules show endophlebitis, which is most conspicuous in the earlier stages of the disease. Inclusion bodies such as are frequently observed in other virus diseases have not been described in epidemic hepatitis. In many respects the lesion of the liver resembles that of idiopathic acute yellow atrophy. Most recent writers<sup>15</sup> on the subject are of the opinion that the hepatic lesion in the fatal case of epidemic hepatitis may be and usually is indistinguishable from so-called idiopathic yellow atrophy. In fact, in those cases of "acute yellow atrophy" in which a known cause can be excluded (i. e., chloroform, carbon tetrachloride, cinchophen, mushroom toxins, phosphorus, arsenic and bacterial toxins) it is probable that the essential disease is epidemic hepatitis. In other words, when a pathologist discovers "idiopathic acute yellow atrophy" at autopsy, the burden of proof is on him to exclude the probability of epidemic hepatitis. In such cases, concurrent lesions in other organs, such as meningoencephalitis, acute regional lymphadenitis, orchitis or acute splenitis, may be of considerable assistance in establishing the diagnosis. The damaged areas are repaired by regeneration (hyperplasia), from residual liver cells located peripherally in the lobules and possibly from the small perilobular (septal) bile ducts, which begins early. In the fatal cases the restitution of normal lobulation (architectural pattern) is seldom perfect, although in cases in which clinical recovery has occurred the anatomic repair is such as to approach closely normal structure, often with no residual changes being evident. Lucke in two recent articles has contributed much to the former meager pathologic knowledge of this disease. In one article<sup>14a</sup> he analyzed the morphologic material obtained by the Army Medical Museum in 125 fatal cases of the outbreak of jaundice which occurred in the Army of the United States during the spring and summer of 1942. The mortality rate in this outbreak was 0.24 per cent. In the second paper<sup>14b</sup> he described the hepatic lesions in 14 people who had recovered from epidemic hepatitis but who died at intervals varying from one week to fourteen months later either of unrelated disease or as the result of an accident. Dibble, McMichael and Sherlock<sup>15a</sup> by aspiration biopsy obtained valuable data referable to hepatic lesions during different stages of the disease in 56 cases.

According to published reports, the mortality from epidemic hepatitis in the United States Navy has been practically nil. Fortu-

15. (a) Dible, J. H.; McMichael, J., and Sherlock, S. P. V.: Lancet 2:402, 1943. (b) Cockayne, E. A.: Quart. J. Med. 6:1, 1912-1913. (c) Lucke.<sup>14a</sup>

nately, in contrast to the army experience of 1942, little "contaminated" yellow fever vaccine, made with human serum, was released to the navy. During the past two years, however, several outbreaks of epidemic hepatitis have occurred at various naval establishments at home and abroad. Willard<sup>16</sup> reported an epidemic occurring at a base hospital with "more than 750 admissions." An outbreak of 320 cases at an unidentified United States naval hospital was reported by Simpson, Powers and Lehman.<sup>17</sup> Logan<sup>18</sup> reported 45 cases from the United States naval hospital in Philadelphia. Cohen<sup>19</sup> at an advance United States naval base hospital reported 360 cases. An outbreak of 73 cases in Tunisia was investigated by Gezon.<sup>20</sup> The fact that in all these epidemics no deaths occurred emphasizes not only the low mortality of the disease but the scant opportunity pathologists have had to study it from the point of view of pathologic anatomy. A group of 30 cases was reported by DeVeer and Matzner,<sup>6</sup> in all but 1 of which recovery occurred. The exception was a case of post-vaccinal (yellow fever) jaundice. The incubation period in this case was approximately four months, and the characteristic lesions of the liver were indistinguishable from those of epidemic hepatitis.

#### MATERIAL

Inasmuch as present anatomic and histologic knowledge of epidemic hepatitis is still rather meager, and in view of the extremely few deaths from this cause reported in the naval service, the addition of a report of other cases, especially of those with unusual features, to the literature seems warranted at this time.

During the past twenty-one months at two naval hospitals in the San Francisco Bay area the author has had the unusual opportunity of making autopsies on 10 persons who died of this disease—all white personnel. Three were encountered at one institution and 7 at another. Although there were a number of patients with "catarrhal jaundice" at each institution their number was not as great as one would have expected from the number of deaths, considering the low mortality rates which have been reported elsewhere to date. This may be due in part, however, to several factors. First, in both hospitals many of the patients were received from ships afloat and from installations overseas, and, second, a number of the patients with the disease had been previously wounded, had received multiple transfusions and had probably suffered considerable loss of nitrogen consequent to their wounds. These men had been wounded three to three and one-half months previously. In this group of wounded men who subsequently contracted hepatitis, the disease seemed to be much more virulent and associated with a higher mortality rate than has been generally observed heretofore. In a group of 32 wounded men in whom jaundice later developed there were 6 deaths, or a mortality of 19 per cent.

16. Willard, J. H. W.: U. S. Nav. M. Bull. **42**:1085, 1944.

17. Simpson, W. M.; Powers, W. L., and Lehman, R. G.: U. S. Nav. M. Bull. **41**:1620, 1943.

18. Logan, V. W.: U. S. Nav. M. Bull. **43**:271, 1944.

19. Cohen, M. I.: U. S. Nav. M. Bull. **43**:1166, 1944.

20. Gezon, H. M.: U. S. Nav. M. Bull. **43**:579, 1944.

These men most probably had "homologous serum jaundice," although proof is lacking.

In view of the facts that in most of the cases in the total series of 10 (see table) death occurred in the early stages of the disease, considerably earlier than heretofore reported in the literature, the mortality was unprecedented, and because a number of the patients probably had "homologous serum jaundice," a rather detailed description of the clinical, anatomic and histologic findings will be presented.

In addition to the classic hepatic changes which were shown in all the cases, there were other features of interest. Nervous and mental manifestations dominated

#### *Summary of Data on Cases in Which Death Was Due to Acute Hepatitis*

Case	Age, Yr.	Duration of Hepatitis (Clinical), Days	Neuro-logic Disturbances	Size of Liver, Gm.	Ascites, Cc.	Size of Spleen, Gm.	Complications	Maximal Interval of Time for Possible Transmission by Transfusion, Months
1	21	7	++++	850	0	211	Wounds, multiple, old (?)	3½
2	24	4	++++	1,000	0	...	Pachymeningitis	4
3	27	2 (?)	..	1,912	?	622	Hemoperitoneum	
4	22	9	++	1,125	0	258	Wounds, multiple, old	3
5*	24	7	++	?	0	?	Wounds, multiple, old	3
6	23	10	++	1,135	50	325	Wounds, multiple, old	3
7	23	6	+	1,475	0	400	Wounds, multiple, old	3½
8	19	6	..	1,727	300	517	Wounds, multiple, old	4
9	28	62 (?)	..	860	1,000	260	Bacterial endocarditis	
10	23	47 (?)	..	1,890	4,600	585	Recovery with recurrence	

\* No autopsy was made. Liver tissue was obtained for biopsy with a Vim-Silverman needle.

the clinical picture in 2 cases. Spontaneous rupture of the splenic capsule with fatal hemoperitoneum occurred in another case on the second day of clinical illness. Six patients had sustained battle wounds three to four months previously. To the best of my knowledge the previously wounded men had not been in contact with persons who had hepatitis. Anatomic material was obtained by autopsy in 9 cases and by biopsy of the liver with a Vim-Silverman needle in another. The initial symptom in 2 cases was abdominal distress which was of such constancy and nature as to render both patients candidates for laparotomy.

#### CASES IN WHICH NERVOUS AND MENTAL MANIFESTATIONS PREDOMINATED

CASE 1.—The patient was a private in the United States Marine Corps, 21 years old. The duration of his hepatitis was seven days. He complained of nausea and vomiting.

The patient had received multiple shrapnel wounds four and one-half months previously, June 27, 1944, while in combat on Saipan. Among these wounds was a compound fracture of the left femur. He was hospitalized, but records of that hospitalization are not available. It seems probable, however, in view of the severity of his wounds, that he must have received transfusions of whole blood or plasma or both. By November 1 he was ambulatory and able to bear his full weight on the injured leg. Three weeks prior to the terminal episode he had an attack of nausea and vomiting, from which he had apparently recovered. The evening of November 17 he became nauseated and vomited.

The results of his physical examination were essentially negative. His temperature, pulse and respirations were normal. The skin and the scleras revealed no abnormal changes of color. There was no abdominal tenderness. Neurologic findings were uniformly negative.

The nausea and vomiting continued at intervals with no accompanying symptoms for four days. During this four day period there were several stools, all of which appeared normal. Early in the afternoon of November 21 he showed transitory improvement and was able to retain liquids. In the evening, however, he became restless and rather irrational. His temperature, pulse and respirations were still normal. The next morning (November 22) the patient was alternately delirious and comatose. Physical findings, as well as the results of examinations of blood and urine, remained essentially negative. In the afternoon there were noted a positive Babinski reflex, generalized hyperactivity of the knee reflexes and absence of superficial reflexes. The fundi were normal, and there was no nuchal rigidity. Spinal fluid findings were all within normal limits except for a slight elevation of the sugar. A sample of blood taken November 22 showed a carbon dioxide-combining power of 37 volumes per cent. Beginning at 5 a. m., November 23, convulsions occurred at intervals, and the patient became feverish. He was hyperventilating to a marked degree. For the first time it was noticed that he was markedly jaundiced. At 9 a. m. he was still hyperpneic, with waves of decerebrate rigidity occurring every five minutes. Rectally the temperature had risen to 107.6 F. Palpation revealed no enlargement of the liver. The blood carbon dioxide-combining power had dropped to 24 volumes per cent and the blood sugar to 70 mg. per hundred cubic centimeters. Therapy consisted of intravenous injections of dextrose, calcium gluconate, nicotinic acid and vitamin B complex. His temperature dropped to 104 F. in the afternoon, and his respirations became nearly normal, although waves of decerebrate rigidity continued to occur. The patient died at 4:20 a. m. November 24.

The clinical diagnosis was cerebrovascular accident (cause in question).

*Pathologic Observations.*—The skin, the scleras, the mucous membranes and the conjunctivas showed a moderate chrome icterus.

The liver was markedly shrunken and weighed 850 Gm. Glisson's capsule was diffusely wrinkled. On cut section the parenchyma was flabby and brownish red, with a loss of lobular markings except for the lateral portion of the left lobe. Here lobular markings persisted. Histologic sections from different areas showed changes typical of so-called idiopathic acute yellow atrophy. Most of the liver cells were absent. Lobular outlines persisted, however, the portal triads being preserved but situated closer to one another than in the normal liver. In subtotally destroyed lobules the autolysis of liver cells involved the central and midzonal areas of the lobules; only the peripheral hepatic cells remained. The sinusoids in the damaged areas were closely approximated, widely dilated and engorged with blood. Small groups of lymphocytes infiltrated the walls of the central venules and focally infiltrated the stroma elsewhere. Fat stains of frozen sections revealed only scant lipoid.

The spleen was slightly enlarged and weighed 211 Gm. Its pulp was firm and dark reddish brown.

Both kidneys were slightly swollen and possessed pale yellowish orange cortices. The left kidney weighed 195 Gm. and the right 187 Gm. Histologic sections revealed extensive fatty phanerosis of the epithelium of the convoluted tubules.

The regional lymph nodes were moderately enlarged and spongy. Microscopically, they were diffusely infiltrated by an admixture of lymphocytes and poly-

morphonuclear leukocytes. Some of their germinal centers were large and edematous and were infiltrated by occasional polymorphonuclear leukocytes.

Sections of the pyloric and fundic portions of the stomach revealed no significant changes. The testes were not examined.

The brain weighed 1,525 Gm. There was considerable congestion of the leptomeninges, which was most marked over the left temporal and occipital regions. A few tiny hemorrhages occurred beneath the ependymal lining of the fourth ventricle. A few tiny petechial spots with variable peripheral discoloration occurred in the mesencephalon rostral to the pons. Several tiny foci of slight softening were detected in the region of the substantia nigra at the level of the mamillary bodies. Histologic sections revealed a few tiny perivascular hemorrhages in the mesencephalon, a slight perivascular lymphocytic infiltration in the tegmentum of the pons and mild meningoencephalitis, which was most evident about the brain stem and the cerebellum. No significant changes were noted in the ganglion cells.

The anatomic diagnosis was: acute epidemic hepatitis with jaundice; mild meningoencephalitis; multiple tiny hemorrhages of the midbrain.

CASE 2.—A chief quartermaster of the United States Navy, 24 years old, had hepatitis for approximately four days. His complaint was pain in the epigastrium, lasting one day.

His past history was as follows: July 5, 1943 he sustained immersion blast injuries after having abandoned ship. While he swam and until he was rescued, four hours later, he suffered much abdominal pain. After his rescue the results of his physical examination were negative except for mild distention and tenderness of the abdomen, which also showed slight generalized rigidity. On July 5 he was given 500 cc. of plasma intravenously. By July 18 the abdominal soreness had disappeared. His recovery was uneventful and he was returned to duty July 21.

Oct. 24, 1943 he reported to the sick bay aboard his ship. He stated that he had experienced slight pain in the "stomach" for one day, and that on the preceding day he felt slightly ill and had a chill, at which time he felt feverish. His temperature, pulse and respirations were normal. He became somewhat nervous, and slight irritation of the throat developed. By the evening of the next day he had become gradually semicomatose and could be aroused only with difficulty. Incontinence developed, and stupor deepened. The pupils were equal but dilated. All the reflexes were normal. October 27 he was transferred to a United States naval hospital. At this time he was comatose and could not be aroused.

His temperature was 101.4 F.; pulse rate, 150; respirations, 40 per minute; blood pressure, 155 systolic and 75 diastolic. He was comatose and appeared critically ill. The skin and the scleras were moderately jaundiced. The pupils were equal and reacted to light. There was mild opisthotonus. Occasionally there occurred convulsive tremors, which were predominantly right sided. The knee jerks were extremely hyperactive. Ankle clonus was marked. Abdominal reflexes and nuchal rigidity were absent. His breathing was stertorous.

On his admission to the hospital at 12:05 a. m. October 27, the spinal fluid was clear and was under a pressure of 150 mm. of water. There were 3 white blood cells per cubic millimeter. The Pandy test was negative. Spinal fluid from a second lumbar puncture nine hours later was xanthochromic. It contained 12 white blood cells and 660 red blood corpuscles per cubic millimeter. The white cell count of the blood was 14,000, with 17 per cent polymorphonuclear leukocytes. A blood smear was negative for plasmodia. Mild right-sided convulsive seizures developed. The patient succumbed thirteen hours after he entered the hospital.

The clinical diagnosis was acute encephalitis.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed moderate icterus. The liver was markedly shrunken and weighed 1,000 Gm. Glisson's capsule was wrinkled, especially over the left lobe. On cut section the parenchyma was found to be flabby, brownish red and slightly edematous. Lobular markings were indistinct. Water-clear bile was contained in the bile ducts. Over the right lobe of the liver anteriorly was a broad depressed scar with a few fibrous adhesions between it and the anterior abdominal wall. Histologically, extensive hepatocellular necrosis and autolysis of liver cells were observed.

Peripherally in a few scattered lobules occasional liver cells remained. The sinusoids appeared widely dilated, closely grouped and engorged with blood. Small groups of lymphocytes were focally distributed throughout. Fat stains of frozen sections revealed no lipid.

The right kidney possessed a rather broad, slightly depressed scar antero-medially, over which the capsule was adherent.

Fibrous adhesions occurred between several loops of ileum and occurred elsewhere in the peritoneal cavity. Multiple purpuric hemorrhages occurred beneath the peritoneum, the pericardium, the endocardium and the mucosal lining of the renal pelvis.

The dura mater showed an area of internal hemorrhagic pachymeningitis over the right parietal lobe with adhesions to an area of the leptomeninges overlying a cortical scar. Histologic sections showed slight fibrous thickening of the dura with occasional presence of pigment-laden phagocytes. Covering the inner aspects of the dura was much recent hemorrhage. The brain showed a rather broad depressed scar in the right parietal lobe immediately beneath the area of pachymeningitis. Histologic sections through this area showed considerable loss of cortical tissue. Microglial cells were increased in number, and there were occasional multinuclear phagocytes laden with brown granular pigment.

The anatomic diagnosis was as follows: acute epidemic hepatitis; ecchymotic hemorrhage of the peritoneum, the pericardium, the endocardium and the renal pelvis; subdural hemorrhage; internal hemorrhagic pachymeningitis; dural-leptomeningeal adhesions; cerebral cortical scar; scars of the liver and the right kidney.

The neurologic signs noted clinically in case 1 cannot be ascribed to any one specific lesion. The demonstrable brain changes were characterized by mild meningoencephalitis and a few tiny hemorrhages occurring in the mesencephalon and in the floor of the fourth ventricle. It seems most probable that the nervous and mental manifestations were on a physiologic rather than an anatomic basis. Characteristically the liver showed lesions typical of acute epidemic hepatitis. In the second case the neurologic disturbances were secondary to a coincidental finding of great interest. It seems probable that this man's prothrombin level had been lowered as a result of the extensive hepatic damage, making conditions favorable for an exacerbation of bleeding from the area of pachymeningitis. The pachymeningitis arising in the absence of the usual predisposing factors (tuberculosis, syphilis and alcoholism) is of extreme interest in view of the immersion blast injury which had been sustained four months previously. The adhesions between the area of pachymeningitis and the underlying scarred cerebral cortex suggest a sudden contusion as the most probable etiologic factor.

The dural and cerebral scars associated with the scars in the right lobe of the liver and the multiple peritoneal adhesions suggest most strongly that the submersion blast injury sustained four months previously had been the common causative factor. Dural and cerebral cortical lesions secondary to immersion blast injuries have not been previously described.

EARLY ACUTE HEPATITIS COMPLICATED BY SPONTANEOUS RUPTURE  
OF THE SPLEEN AND FATAL HEMOPERITONEUM

In the past there has been little opportunity to study the liver, the spleen and other tissues during the early stages of acute epidemic hepatitis. In the army series of 125 cases<sup>14a</sup> the patient showing the earliest stage at autopsy had survived ten days. In this regard Lucke stated: "None of the livers in this series were in the early stages of destruction; indeed so far as I have been able to learn from the literature, no one has ever seen the earliest stage in this disease, which rarely terminates in its most acute stages." Not only has knowledge of the early stages of the hepatic changes been scanty but especially information pertaining to early changes in the spleen and other organs. Most of the data available on early hepatic changes have been obtained by aspiration biopsy.<sup>15a</sup> Few patients with this disease die in the preicteric and intermediate phases. For this reason there is little available knowledge referable to the state of the spleen as well as of other organs in the initial stages of the disease. According to the scant data now in the literature, the spleen is soft for a short while in the early stages of involvement. Later, however, it is uniformly firm and usually enlarged to a moderate degree. In the case to be reported the clinical duration of hepatitis is estimated to have been not more than two days. The spleen was enlarged and extremely soft. A tiny laceration of the splenic capsule overlying a subcapsular hemorrhage resulted in a fatal hemoperitoneum. Unfortunately, it was not possible to obtain data on the prothrombin level in this case. It seems probable in view of the histologic observations, however, that splenitis, rather than a low level of prothrombin, was an important factor in initiating the subcapsular hemorrhage. The acute interstitial pneumonitis, myocarditis and orchitis concurrent with the splenitis and the hepatitis are indicative of a virus origin.

CASE 3.—The patient was a pharmacist's mate 27 years old. The duration of the hepatitis was estimated to have been not over two days. The complaint was fatigue for possibly two days, with jaundice developing in one day.

The patient had never been outside of the United States and had had no serious illnesses. Four weeks prior to the hepatitis he had an acute febrile illness which was characterized by a morbilliform eruption and posterior cervical lymphadenopathy. This illness was diagnosed as "German measles." He was hospi-

talized one week and was returned to duty around November 20. On December 4 he departed with a draft of patients to Great Lakes, Ill. En route, a fellow corpsman became ill, jaundice developed, and the man had to be removed from the train to a hospital in Chicago. This man died a few days later and the necropsy was reported as showing "idiopathic acute yellow atrophy of the liver." On the return trip by train the pharmacist's mate complained of fatigue and on the last day of the journey noticed slight jaundice. He became progressively weaker, and an ambulance was ordered to meet the train. The patient died in the ambulance December 16 while en route to the hospital.

*Pathologic Observations.*—The skin and the scleras were pale lemon yellow. The peritoneal cavity contained 2800 cc. of fluid blood.

The liver was slightly enlarged and weighed 1,912 Gm. Glisson's capsule was smooth and pale chocolate brown, and the edges of the liver were slightly rounded. The hepatic parenchyma was slightly opaque, yellowish orange and ischemic, and its lobular markings were indistinct. Histologic sections revealed extensive stromal and parenchymatous changes. Many of the liver cells were swollen, and all were detached from one another in varying degrees, frequently disrupting the continuity of the hepatic cell cords (fig. 1A). Even though these changes were diffuse, they seemed more accentuated in the central and midzonal portions of the hepatic lobules. Not infrequently the biliary canaliculi were slightly dilated and contained yellowish brown bile. Infiltrating the stroma were prominent groups of lymphocytes and mononuclear phagocytes. Fat stains of frozen sections revealed a scant amount of lipid. Some of the swollen hepatic cells contained large vacuolated oval nuclei. No inclusion bodies were seen.

The spleen was greatly enlarged, soft and flabby. It weighed 622 Gm. and measured 21.5 by 12 by 3.5 cm. Medially along the convexity of the lower third the capsule was lacerated over an area measuring 1.5 by 2 cm. Immediately beneath and about the laceration was a large subcapsular hemorrhage which measured 8 by 10 cm. Otherwise, the capsule was smooth and grayish purple. The pulp was semiliquid, pale grayish brown and quite opaque. Histologically, the malpighian bodies were small, compact and discrete. The pulp was extremely cellular, being infiltrated by a myriad of lymphocytes. Prominent areas of edema occurred in places (fig. 2A).

The testes were grossly normal but histologically showed moderate to marked arrest of spermatogenesis. Infiltrating the testicular stroma were a few widely scattered small groups of lymphocytes (fig. 2B).

The heart microscopically showed myocarditis. Small groups of lymphocytes with occasional mononuclear phagocytes infiltrated the myocardium between the muscle fibers of the trabeculae carneae and also were prominent immediately beneath the endocardium.

The lungs were firm but aerated throughout. Histologically, the pulmonary alveolar walls were moderately thickened because of edema and moderate numbers of infiltrating lymphocytes and mononuclear phagocytes. Similar cells also formed moderate perivascular collars. Most of the alveoli were empty except for a few pigment-laden phagocytes.

The regional lymph nodes, mainly the mesenteric, the retroperitoneal and those about the celiac axis, were considerably enlarged, edematous and hyperemic. Histologically, all showed moderate lymphoid hyperplasia and lymph sinuses engorged with moderate numbers of lymphocytes and mononuclear phagocytes. One node showed prominent foci of necrosis and autolysis. In most of the nodes the interfollicular lymphoid tissue was especially abundant.

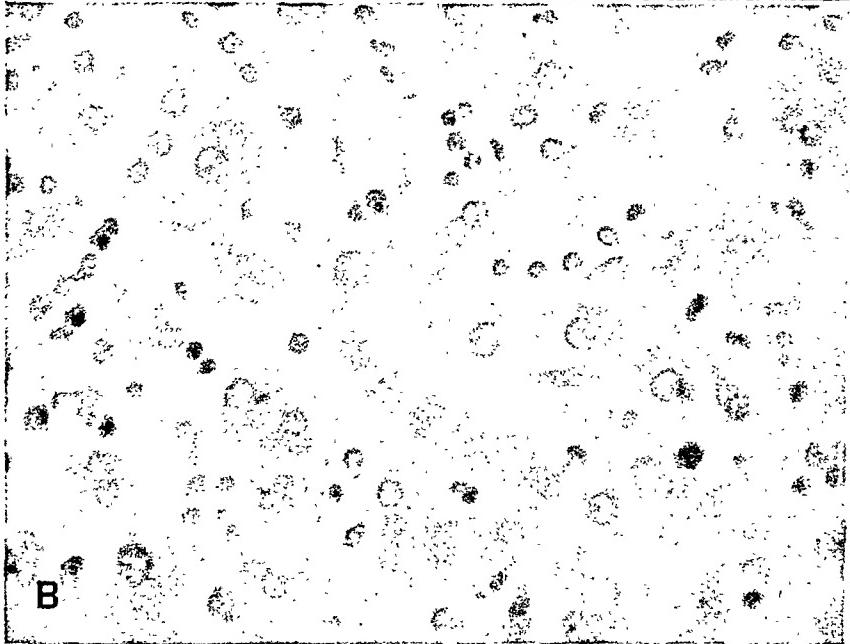
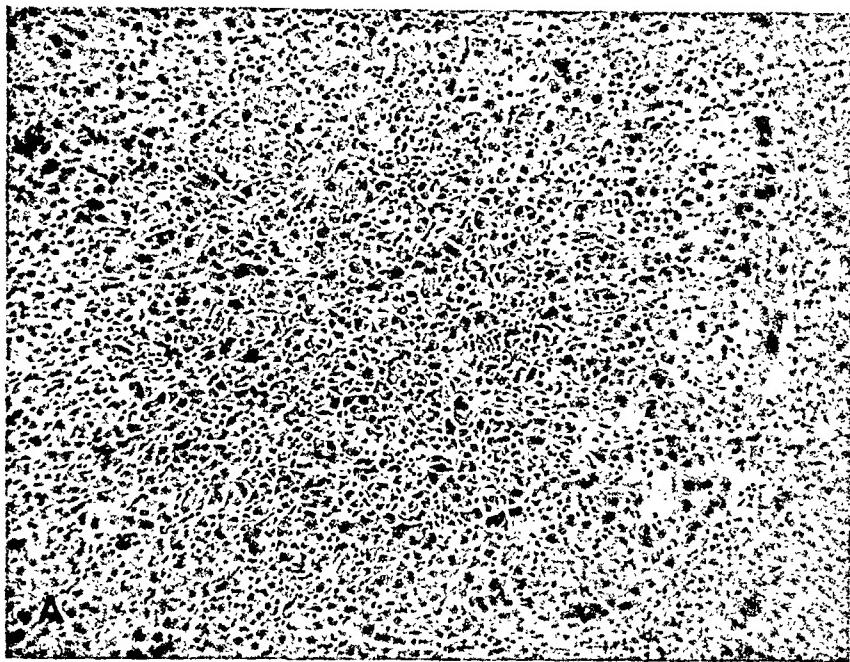


Fig. 1.—*A*, liver in case 3. Many of the liver cells are swollen. Nearly all are detached from one another, frequently disrupting the continuity of the hepatic cell cords. Prominent numbers of lymphocytes and monocytes diffusely infiltrate the stroma. Changes are most marked in the central and midzonal areas. ( $\times 50$ .)

*B*, higher magnification of *A*. ( $\times 550$ .)



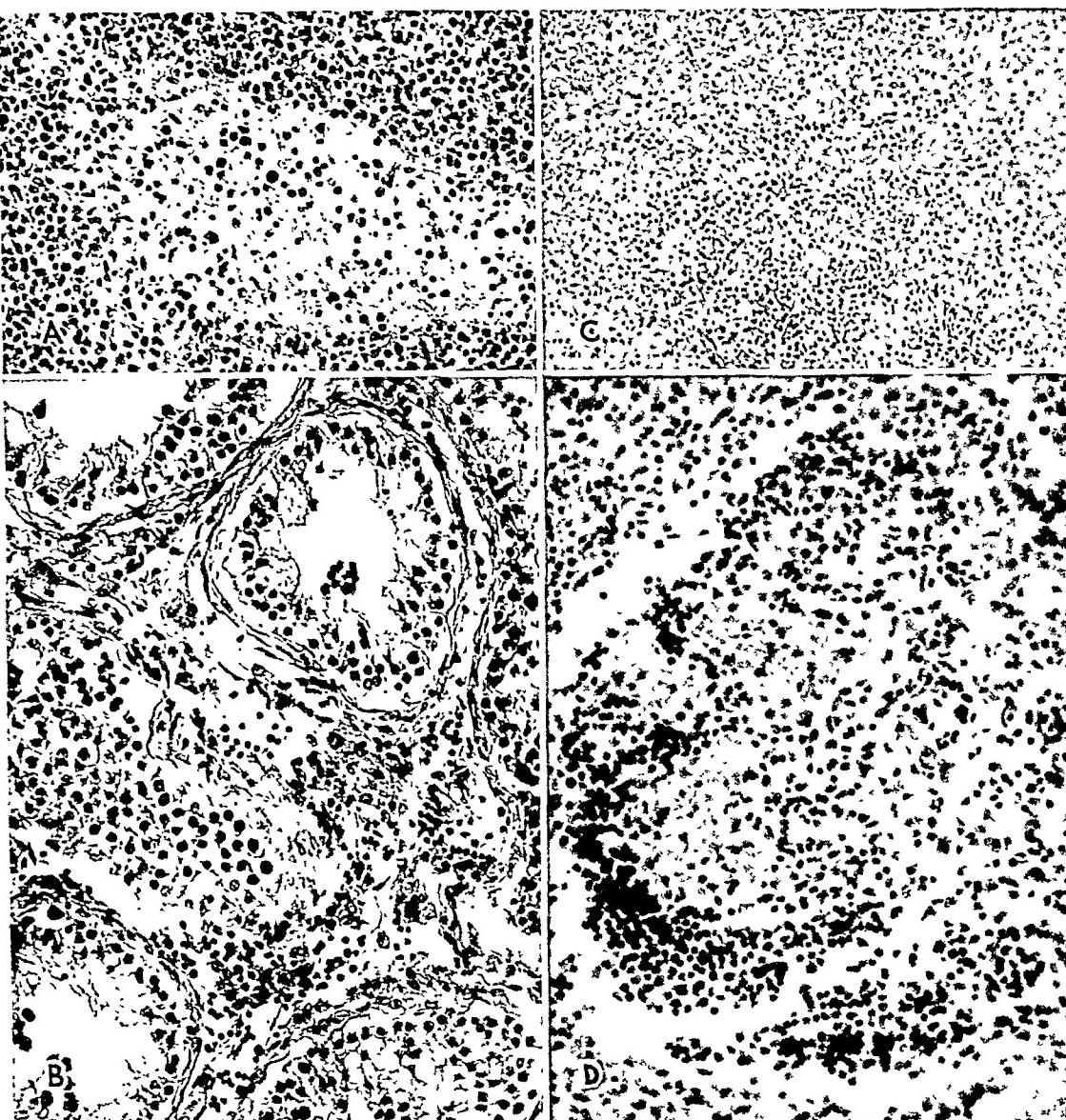


Fig. 2.—*A*, spleen in case 3. Centrally there is a large area of edema. Note the marked lymphocytic infiltration of the adjacent tissue. ( $\times 85.$ )

*B*, orchitis with arrest of spermatogenesis in case 3. Moderate numbers of lymphocytic cells infiltrate the stroma. The spermatic tubules are devoid of healthy sperm. ( $\times 85.$ )

*C*, liver in case 7. Diffuse necrosis and autolysis of liver cells involves all except a few of the peripheral lobular cells. Autolysis is not complete. Dead remaining liver cells and cellular debris are stained prominently with eosin. Throughout is a diffuse lymphocytic infiltration of the stroma. ( $\times 122.5.$ )

*D*, lymph node from the hilus of the liver in case 7. Necrosis of a germinal center is associated with a mild infiltration of polymorphonuclear leukocytes. ( $\times 234.$ )

The common bile duct contained thin, pale orange bile and showed no anatomic abnormalities. The portal vein was intact and contained thin fluid blood.

The anatomic diagnosis was as follows: acute epidemic hepatitis; acute splenitis; subcapsular splenic hemorrhage with laceration of the capsule; hemoperitoneum (2,800 cc.); acute regional lymphadenitis; acute interstitial pneumonitis; orchitis; myocarditis; jaundice; mild fatty degeneration of the kidneys.

This is not only the first case to my knowledge in which epidemic hepatitis was complicated by spontaneous rupture of the spleen but the first one in which death occurred on the second day of illness, at a much earlier stage of the disease than has been previously described. The only hemorrhagic phenomenon which had occurred was the subcapsular splenic hemorrhage. Unfortunately, it was not possible to obtain data on the prothrombin level. It is thought that the hemorrhagic phenomena so frequently seen in the later stages of this disease are most probably related to diminution of the formation of prothrombin by the damaged liver. In this case, however, it seems probable that the underlying factor in the production of the subcapsular hemorrhage may well have been the splenitis which was demonstrated histologically. The hepatic changes shown in this early phase of the disease are of interest. Even though there was evidence of extensive hepatocellular damage, autolysis was still minimal. Of interest were the occasional plugs of bile within the biliary canaliculi. Such plugs have also been noted by others in those few cases in which early changes have been studied either at postmortem examination or at aspiration biopsy. It is thought that this mechanical obstruction of the intralobular canaliculi may be a most important cause of persistence of jaundice. Testicular changes characterized by arrested spermatogenesis have been noted quite commonly but not universally by other writers, and it has been concluded that the hepatic damage must be not only severe but extensive and long standing. It is thought that arrest of spermatogenesis in cases of hepatic liver damage is due to failure of the liver to destroy estrogens circulating in the blood. Yet in the present case the known illness had lasted only two or three days and in addition to the arrest of spermatogenesis there was definite orchitis (fig. 2B). Swollen regional lymph nodes are quite characteristic, particularly in those cases with a duration of less than one month.<sup>14a</sup> It has been thought that the edema, the acute hyperplasia and the acute lymphadenitis may be representative of a reaction secondary to the destruction of large areas of the liver. In this particular case the concomitant acute splenitis and acute interstitial pneumonitis, along with the other lesions, is highly indicative of a virus disease. In the various reports on hepatitis which I have seen this is the first in which interstitial pneumonitis has been described as outstanding and seemingly a part of the systemic disease. Descriptions of splenic lesions in such early stages of hepatitis are rare, and in no

instance described had the splenic changes been as pronounced as in this case. Histologically, there was true splenitis with focal areas of edema. Splenic changes of the same order but less pronounced were observed in cases 7 and 8, in which also relatively early lesions of hepatitis were observed.

FATAL ACUTE EPIDEMIC HEPATITIS OCCURRING IN WOUNDED  
MEN IN WHOM THE ICTEROGENIC AGENT WAS POS-  
SIBLY INTRODUCED BY TRANSFUSIONS OF  
WHOLE BLOOD OR PLASMA

Five of our patients showed a remarkable coincidence of history in that all 5 had been wounded three months previously on the same battlefield, had received multiple transfusions of blood and plasma, had been in contact with no one known to have hepatitis and had become ill at about the same time. A sixth (case 1) had also been wounded three and a half months previously and undoubtedly had been given transfusions but is not included in this group inasmuch as records of treatment at the advanced base hospital where he was originally treated are not available.

The acute hepatitis following transfusions of whole blood, plasma, convalescent measles serum and biologic products containing human serum (the original yellow fever vaccine used early in 1942) is well recognized in the literature. It is felt by the majority of workers that it is in reality produced by a filter-passing icterogenic agent which is closely related to, if not identical with, that which is the cause of acute epidemic hepatitis.

Inasmuch as the 5 patients whose cases are to be reported in the following pages had not been in known contact with persons presenting clinical jaundice, and inasmuch as the intervals of time which elapsed after the first blood transfusions following their wounds averaged three months, it seems probable that the icterogenic material in their cases was introduced intravenously. Clinically, the hepatitis of these patients was striking in the absence of the usual preicteric phase, the short duration and the severity once the disease had become manifest.

The hepatic damage was of the same type in all and classically was that of acute epidemic hepatitis, producing a histologic picture similar to that seen in so-called acute yellow atrophy. Acute splenitis, regional lymphadenitis, orchitis and variable but mild cholemic nephrosis occurred in the majority. Cerebral lesions were minimal, variable and characterized chiefly by small perivascular hemorrhages of focal distribution.

CASE 4.—This patient was a private first class in the United States Marine Corps, 21 years of age. The duration of his hepatitis was nine days. His complaint was multiple wounds of the face with stenosis of the mouth, lasting three months, and nausea and vomiting, one day.

The patient was wounded on Iwo Jima Feb. 28, 1945, and was admitted to the United States Naval Hospital, Oakland, Calif., June 1. He had suffered a loss of the greater portion of his lower and upper jaws and part of his nose. After his injury he had been given many units of whole blood and plasma. At the time of his arrival here the opening of the mouth had closed down with scar tissue so that it was stenotic, consisting of a circular hole approximately one-half inch in diameter. On the second day after his admission he became nauseated and vomited.

The rectal temperature was 100 F., the pulse rate 76, and the respirations 18 per minute. Until the onset of nausea and vomiting physical examination gave essentially negative results except for deformity of the face, stenosis of the mouth, considerable loss of weight and moderate dehydration. He was ambulatory and remarkably cheerful in view of the extent of his injury.

Nausea and vomiting continued in spite of intravenous therapy with dextrose, amigen sol, Betalin Complex<sup>21</sup> and thiamine hydrochloride. In view of the constant nausea and vomiting it was recognized that the stenotic oral opening imposed a constant risk of aspiration pneumonia. The liver and the spleen were at no time palpable. There was consistently mild leukopenia. On June 4 the white blood cell count was 5,550 cells per cubic millimeter, 59 per cent being polymorphonuclear leukocytes. Two days later, it was 4,850, with 55 per cent leukocytes, and the icterus index was 121.7 units. The serum protein on June 4 was 6.5 Gm. per hundred cubic centimeters, and the blood sedimentation rate was 2. On the evening of June 8 the patient lapsed into coma. His vomiting ceased after a period of extreme restlessness and delirium. The following morning he was in deep coma. His temperature was 99 F., pulse rate 100, respirations 10 per minute and blood pressure 130 systolic and 90 diastolic. The abdomen was soft; the liver and the spleen continued to be nonpalpable. Both lower extremities were somewhat spastic, with hyperactive reflexes. There had developed since the preceding evening bilateral ankle clonus, and the Babinski reflex had become positive. The abdominal and cremasteric reflexes were absent. His blood serum had a definite greenish color; due possibly to biliverdin. By the morning of June 10 his temperature had risen to 107 F. rectally; his respirations had become labored and their rate was up to 48; the pulse rate was 150. The abdomen continued to be soft. Several convulsions occurred at intervals. The pupils were dilated, the right arm was spastic, the legs were flaccid, and the Babinski reflex had now become negative. Deep reflexes were not elicitable. The prothrombin percentage had dropped to 27. He remained in coma and died at 4 p. m.

The clinical diagnosis was acute infectious jaundice with possible free intracranial bleeding.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed moderate chrome yellow icterus. The liver was moderately shrunken and weighed 1,120 Gm. It was flabby and possessed a slightly wrinkled, pale brown capsule. Flabbiness was especially marked in the left lobe. On cut section the parenchyma showed a pale tan, ischemic, mottled surface in which it was thought that lobular markings were still evident. There were a few areas, however, where the tissue was purplish red except for small oval islands of pale tissue measuring up to 0.3 cm. Except for a few small islands histologic sections showed almost complete ablation of liver cells. Those liver cells which persisted were perilobular

. 21. Betalin Complex (Vitamin B Complex, Lilly) contains in 2 cc. 10 mg. of thiamine hydrochloride, 4 mg. of riboflavin, 150 mg. of nicotinamide, 5 mg. of pantothenic acid (as calcium pantothenate) and 10 mg. of pyridoxine hydrochloride (vitamin B<sub>6</sub> hydrochloride).

in distribution. Generally, hepatocellular necrosis and autolysis of liver cells dominated the histologic picture. Occasional small groups of lymphocytic cells infiltrated the stroma.

The spleen was slightly enlarged, weighed 258 Gm. and showed moderate hyperemia. Its pulp was firm, brownish red and slightly opaque, and its stroma was focally infiltrated by a few small collections of lymphocytes, phagocytes and polymorphonuclear leukocytes. Malpighian bodies were distinct, and histologically a few were infiltrated by polymorphonuclear leukocytes. Pale brown bile was contained in the extrahepatic biliary passages and in the gallbladder.

Each lung showed moderate aspiration pneumonia.

The brain revealed no gross or histologic lesions of significance except hyperemia.

The testes showed histologically mild but definite arrest of spermatogenesis.

The anatomic diagnosis was acute epidemic hepatitis, mild arrest of spermatogenesis, splenitis, lymphadenitis of the original nodes and bilateral aspiration pneumonia.

**CASE 5.**—A gunnery sergeant in the United States Marine Corps, 24 years of age, had hepatitis for seven days. His complaints were chilliness, nausea and vomiting lasting four days and jaundice one day.

The patient had been wounded in the abdomen on Iwo Jima March 2, 1945. His abdomen was opened three hours later, and three jejunal perforations were sutured. He was given whole blood and penicillin, and other supportive treatment was used. He was evacuated via several naval activities to the United States Naval Hospital, Oakland, Calif., where he was admitted for the first time May 2, 1945. His condition was good, the wound had ceased draining, and on May 12 he was given a week's convalescence leave and transferred to a convalescence hospital on June 8, at which time he felt well. Three days later, however, a temperature of 100.2 F. was recorded, and he complained of chilliness, nausea and vomiting. An early stage of intestinal obstruction was suspected, and he was treated with sulfadiazine, and other general measures were instituted. The symptoms continued until June 15, when icterus appeared and he was transferred as a readmission patient to this hospital with the diagnosis of probable intestinal obstruction.

His temperature was 97.6 F., pulse rate 120 and respiratory rate 24. The skin, the scleras and the mucous membranes showed moderate icterus. The patient was restless and disoriented and did not respond to questions. Otherwise the physical examination showed only distention of the urinary bladder to the level of the umbilicus and suggestive bilateral positive Babinski reflexes.

His treatment consisted of intravenous injections of dextrose, amigen, vitamin B complex and choline, with supportive measures. His icterus index was 104, and the reaction to the cephalin cholesterol flocculation test was 3 plus. On June 17 his prothrombin percentage was 45.5. His delirium and semicomatoso condition persisted. On June 18 he lapsed into deep coma and died. For a day prior to death the following signs were noted: The pharyngeal reflex was absent; the biceps and achilles tendon reflexes were not obtained; the knee jerks were hypoactive; superficial reflexes were absent, and there was no response to painful stimuli.

The clinical diagnosis was acute infectious hepatitis due possibly to serum transmission of the icterogenic agent.

***Pathologic Observations.***—Liver tissue obtained for biopsy with a Vim-Silverman needle revealed the classic microscopic changes of acute epidemic hepatitis. There had occurred extensive autolysis of the central and midzonal liver cells. Those which persisted peripherally in the lobules were quite frequently swollen, were detached from one another and contained finely vacuolated cytoplasm. Peripherally

in the lobules biliary canaliculi were occasionally dilated and contained yellowish plugs of bile. Very occasionally small groups of lymphocytes with a few polymorphonuclear leukocytes infiltrated the supporting stroma. There was preservation of the sinusoids and the stroma. The wall of one central venule had been infiltrated by numerous lymphocytes. In the areas of greatest damage Kupffer cells were prominently laden with lipochrome pigment.

The anatomic diagnosis was acute epidemic hepatitis.

CASE 6.—The patient was a private first class, white, in the United States Marine Corps, 22 years of age. The duration of his hepatitis was seven days.

He was admitted to the United States Naval Hospital, Oakland, Calif., June 10, 1945, at which time his condition was good except for afternoon elevation of temperature and anorexia. March 3, 1945 he had been wounded on Iwo Jima, at which time he had sustained a perforation of the cecum and a wound of the right buttock. The cecum was exteriorized, following which a retroperitoneal abscess developed.

His temperature was 100 F., pulse rate 100 and respiratory rate 18. His general condition was good. There were present a healed bullet wound of entrance in the upper part of the right buttock and a functioning cecostoma in the right iliac region at the upper margin of a granulating incision. An opening in the lower part of the incision communicated with a large abscess in the right iliac fossa and a draining sinus extending to a small wound in the right flank.

The anorexia which the patient had on admittance persisted. Eight days later, on the morning of June 18, the patient suddenly lapsed into severe shock and coma. His pulse was rapid and his respirations were shallow. Slight jaundice of the scleras was noted for the first time. The abdomen was soft and not tender. Supportive treatment was given, with slight transient improvement. A neurologic examination revealed some motion with painful stimuli, a slow bilateral ankle clonus and a bilateral positive Babinski reflex. Sucking and grasping reflexes were absent. The liver and the spleen were not felt. A diagnosis was made of acute atrophy of the liver, probably infectious hepatitis. Dextrose, amigen, Betalin Complex,<sup>21</sup> thiamine hydrochloride and nicotinic acid were given intravenously. His general condition continued to be poor. Deep reflexes in the lower extremities were no longer elicitable, and the patient died at 1:10 a. m., June 20.

The clinical diagnosis was acute yellow atrophy of the liver, most probably acute epidemic jaundice.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed mild icterus. Approximately 50 cc. of amber fluid with flecks of fibrin was contained in the peritoneal cavity. Within the right pleural cavity was 1,000 cc. of clear amber fluid.

The liver was small, shrunken, and weighed 1,135 Gm. Glisson's capsule was pale, purplish brown and relatively smooth except for a few slightly wrinkled and pitted depressed areas over the main lobe anteriorly. On cut section it showed a variegated tannish yellow to dark brownish red pattern, in which lobular markings were thought to be grossly evident (fig. 3A). There was much flabbiness of the left lobe. Histologically, the hepatic changes were classically characteristic of acute epidemic hepatitis. Except for a few, widely scattered, tiny islands of residual liver cells, there had been almost complete hepatocellular necrosis, but with preservation of lobular outlines. Small perilobular bile ducts were numerous, occurred in small groups and were more closely approximated to one another than usual as a result of extensive hepatocellular necrosis with "collapse" of lobules. Small groups of lymphocytes irregularly infiltrated the stroma as well as the walls of some of the central venules. Occasional phagocytes and reticuloendothelial cells



Fig 3.—*A*, liver in case 6. Grossly, the liver was small, shrunken, and weighed 1,135 Gm. There was no nodulation. The freshly cut surface showed a diffuse variegated appearance which suggested accentuated lobular markings.

*B*, liver in case 7. Faint outlines of necrotic liver cells with "coagulated" cytoplasm remain. Such cells are eosinophilic. Autolysis and removal of cellular debris is complete. ( $\times 550$ .)



were prominently laden with yellowish lipochrome pigment. The lymph nodes at the hilus of the liver were large, spongy and pale tan. Small groups of cells, consisting of an admixture of lymphocytes and polymorphonuclear leukocytes, infiltrated the stroma and the lymph sinuses as well as a few germinal centers.

The spleen was moderately enlarged, weighed 325 Gm. and measured 17 by 10.5 by 4.5 cm. On cut section, the malpighian bodies were distinct, and the intervening pulp was firm but dark grayish brown (fig. 4). The latter was infiltrated by scattered small groups of lymphocytes and polymorphonuclear leukocytes. Terminal bilateral bronchopneumonia was associated with a serous effusion of 1,000 cc. in the right pleural space.

The testis, in addition to showing considerable arrest of spermatogenesis, showed mild infiltration of the stroma in the form of small focal collections of lymphocytes.

The brain showed numerous markedly congested small blood vessels in the white matter of the cerebrum, in the basal nuclei and the mesencephalon. Histo-



Fig. 4.—Spleen in case 6; weight 325 grams. The malpighian bodies are distinct. At autopsy the pulp was firm but dark grayish brown.

logically, aside from marked congestion of small blood vessels and occasional tiny perivascular hemorrhages, there were no significant lesions.

The anatomic diagnosis was acute epidemic hepatitis, acute splenitis, bronchopneumonia, pleural effusion, regional lymphadenitis, marked arrest of spermatogenesis, orchitis and gunshot wound of the abdomen.

CASE 7.—The patient was a sergeant of the United States Marine Corps, 23 years of age. The duration of his hepatitis was six days. He complained that he had suffered from a wound of the back with laceration of the ascending colon and a pelvic abscess for three and a half months and from nausea and pain in the upper abdominal region for one day.

He was admitted to the United States Naval Hospital, Oakland, Calif., June 10, 1945, having been wounded on Iwo Jima March 17, 1945. A bullet had entered the right side of the back just above the sacroiliac joint and had traversed the abdomen, lacerating the ascending colon, and having its point of exit just above

the anterior superior spine of the right ilium. Two days later the abdomen was surgically explored, and the lacerated ascending colon was exteriorized. The post-operative course was stormy, with signs of peritonitis. Improvement began about the tenth postoperative day. After operation he received intravenously dextrose, blood plasma and whole blood. April 5 an external closure of the colostoma was performed, and the subsequent course was satisfactory. The patient was transferred to a naval hospital in the Hawaiian Islands April 15, and April 27 a large retroperitoneal abscess on the right side was drained. He was transferred to the United States Naval Hospital, Oakland, Calif., by air June 10. On admission here examination showed an emaciated man complaining of two painful fecal fistulas, one the old colostoma, and the other a wound in the right lateral abdominal wall. He was febrile, with a daily temperature of 101 F. Roentgen studies revealed a large retrocecal abscess extending into the pelvis and communicating with the ascending colon. Determination of the serum protein showed 5.8 Gm. per hundred cubic centimeters. June 22 a transfusion of 100 cc. of whole blood was given, and the next day transverse ileocolostomy was performed with the patient under cyclopropane-oxygen anesthesia. Following this procedure, he was given 20,000 units of penicillin every two hours for the next six days. He became afebrile immediately after the operation, and his general condition improved July 5, when he complained of nausea, pain in the upper abdominal region and fever, his temperature rising to 104 F.

On physical examination his temperature was 102.4 F., pulse rate 95 and respiratory rate 20. The scleras, the skin and the mucous membranes showed mild icterus. There was diffuse tenderness of the abdomen to deep palpation but no abdominal rigidity.

The patient was given intravenously dextrose, vitamin B complex and amigen. At no time did the liver or the spleen become palpable. The white blood cell count varied from 10,750 to 15,200, with a normal differential count. The reaction to the cephalin-cholesterol flocculation test was 4 plus. The icterus index rose to 28.1 July 9. The prothrombin time on this date was 88 per cent of normal, and the urine revealed no bile. His general condition was fair. His temperature continued between 103 and 104 F. until July 10, when it dropped to 94.2 F. rectally. He became comatose, irrational and restless. Neurologically, there were generalized motor weakness and absence of reflexes. He died on the following day, July 11, in circulatory collapse.

The clinical diagnosis was acute infectious jaundice.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed mild icterus. The liver weighed 1,475 Gm. In the left lobe, there was much flabbiness of the parenchyma, and the overlying Glisson's capsule was slightly wrinkled—otherwise the capsule was smooth. On cut section, lobular markings persisted, and there was present a uniform mottled appearance with narrow tannish yellow streaks of tissue surrounding dark brownish red oval islands centrally. Histologically, diffuse changes were characterized by subtotal necrosis and autolysis of the liver cells. There persisted in scattered foci outlines of "coagulated" liver cells with no nuclear staining (figs. 2C and 3B). The presence of such "dead" cells indicated incomplete removal of cellular debris in contrast to many of the other cases, in which the process had been completed.

The spleen was moderately enlarged and weighed 400 Gm. It was flabby, and on cut section its pulp was slightly opaque, rather soft and brownish red. The malpighian bodies were small and faintly visible through the slightly swollen, opaque surface. Microscopically, however, the malpighian bodies were distinct.

Many showed much edema centrally and were infiltrated in such areas by moderate numbers of poorly preserved polymorphonuclear leukocytes. Occasional malpighian bodies showed necrosis of the germinal centers. Many small groups of lymphocytic cells with occasional polymorphonuclear leukocytes and mononuclear phagocytes infiltrated the pulp.

The testes were grossly normal but histologically showed moderate to marked arrest of spermatogenesis. Occasional small groups of lymphocytes infiltrated the stroma, and about such areas there was mild edema.

The regional lymph nodes were markedly enlarged, measuring up to 3.5 cm. in width, and were edematous. On cut section they had a swollen, mottled tan appearance. Microscopically, the lymphoid follicles were large, and a number of their swollen germinal centers contained small foci of necrosis (fig. 2D). Other germinal centers were epithelioid in appearance and bounded by only a narrow zone of lymphocytic cells. The lungs were normal except for a small tannish white area in the left lung, which microscopically showed interstitial pneumonitis of moderate degree. The brain was essentially normal except for marked congestion of small blood vessels in the white matter of the cerebrum, in the basal nuclei and in the mesencephalon.

The anatomic diagnosis was: acute epidemic hepatitis; acute splenitis; acute lymphadenitis of the regional lymph nodes; interstitial pneumonitis; orchitis with arrest of spermatogenesis; multiple old wounds of the abdomen.

CASE 8.—The patient was a private first class of the United States Marine Corps, 19 years of age. The duration of his hepatitis was six days. He complained that he had suffered from multiple shrapnel wounds of the pelvis four months and jaundice one day.

This patient was admitted to the United States Naval Hospital, Oakland, Calif., April 14, 1945, having been wounded on Iwo Jima Feb. 19, 1945. He sustained multiple wounds of the pelvis, resulting in a ureterorectal fistula, two cutaneo-vesicular fistulas and an injury to the lower part of the bowel. As a consequence of these injuries suprapubic cystostomy and colostomy were performed. The postoperative course and convalescence were slow but progressive. June 4 the patient began to complain of pain of the upper part of the abdomen. Because of the persistence of the abdominal pain it was thought that hepatitis might possibly be developing, and tests were made: The icterus index June 14 was 6.3 units, and the cephalin-cholesterol flocculation test gave a result of 2 plus June 15 and June 17. For the next month the patient had no complaints, and his general condition changed very little. July 11, however, he became deeply jaundiced and drowsy but was mentally clear.

There were no unusual findings on physical examination except for hyperactive reflexes and bilateral ankle clonus. His temperature was 98 F., pulse rate 100 and respiratory rate 20. The scleras, the skin and the mucous membranes showed deep icterus.

The patient was given intravenously dextrose, amigen sol, Betalin Complex,<sup>21</sup> thiamine hydrochloride, and nicotinamide. The following three days his condition remained about stationary. He then became drowsy, and his temperature varied from 101 to 103 F. He was given a transfusion of whole blood but failed to improve. He remained mentally alert. July 16 moderate respiratory distress developed, and a few coarse rales were heard at the base of each lung. Chills also occurred, and the next day the patient suddenly died.

The clinical diagnosis was acute hepatitis.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed moderate chrome yellow icterus.

The liver was normal in size and weighed 1,727 Gm., but was flabby in consistency. Glisson's capsule was pale brown and slightly wrinkled over the posterior aspect of the lesser lobe, which appeared considerably less thick than normal. Beneath the capsule, the lobular markings appeared distinct, even accentuated, and there was a definite mottled appearance with small, oval, reddish brown islands surrounded by pale yellowish parenchyma. This appearance was not uniform, however, inasmuch as large, opaque, swollen, grayish brown areas in the dome of the main lobe showed complete absence of lobular outlines. Histologically, there was extensive autolysis of liver cells, which involved chiefly the midzonal and central lobular areas. Moderate numbers of liver cells remained, and these were large, swollen and stained poorly. Rather numerous lymphocytes diffusely infiltrated the stroma of the lobules and the walls of the central venules. Among these were occasional polymorphonuclear leukocytes. A moderate number of Kupffer cells were laden with yellowish lipochrome pigment.

The spleen was moderately enlarged and weighed 517 Gm. Its capsule was tense and tore readily on removal of the organs. The pulp was slightly edematous, semiliquid, dark brownish purple and slightly opaque. Malpighian bodies were prominent. Microscopically, there was extreme congestion of the pulp. The malpighian bodies were rather indistinct, and a number showed necrosis of their centers.

The testes were rather small, weighing 13 and 16 Gm., respectively, and microscopically showed marked diffuse arrest of spermatogenesis with a mild degree of orchitis, characterized by tiny focal infiltrations of lymphocytes.

The lymph nodes at the hilus of the liver were greatly enlarged and spongy and measured up to 4.0 cm. in width. Histologically, they showed moderate to marked edema. The germinal centers of the lymphoid follicles were indistinct, and a few showed mild necrosis accompanied by a mild to moderate infiltration of polymorphonuclear leukocytes.

The brain was essentially normal except for marked congestion of small blood vessels in the basal nuclei, the mesencephalon and the white matter of the cerebrum.

*Ecchymotic hemorrhages* occurred beneath the epicardium and the endocardium.

The anatomic diagnosis was acute epidemic hepatitis; acute splenitis; acute lymphadenitis of the regional lymph nodes; orchitis with arrest of spermatogenesis; multiple old wounds of the pelvis with a rectoureteral fistula and pyelonephritis.

An interesting feature of this case is the fact that the cephalin-cholesterol flocculation test was positive on two occasions one month before the advent of clinical jaundice. This observation suggests the value of this test when obscure abdominal complaints, even though minor, develop in previously wounded men. In a companion paper Snell, Wood and Meienberg<sup>22</sup> pointed out that this test is of value in detecting the presence of hepatic damage in the incubation period and the preicteric phase. They pointed out further that when the test is positive under such circumstances the administration of gamma globulin may be of distinct value at that time in attenuating or aborting clinical development of the disease.

22. Snell, A. M.; Wood, D. A., and Meienberg, L. V.: Infectious Hepatitis with Especial Reference to Its Occurrence in Wounded Men, *Gastroenterology* 5: 241, 1945.

## MISCELLANEOUS CASES OF EPIDEMIC HEPATITIS

The last 2 cases in this series conform more closely to the recognized clinical picture of epidemic hepatitis, especially as regards duration of the disease. In contrast to the preceding 5 cases, the clinical course in each of these cases (in retrospect) showed well defined preicteric, intermediate and final phases. Even though the symptoms were classically referable to the abdomen, these 2 cases presented diagnostic difficulties, as well as interesting necropsy findings. In 1 case the condition seemed to be improving clinically when a sudden abdominal episode, most probably secondary to an unrecognized intercurrent attack of bacterial endocarditis, caused a critical turn for the worse, with death occurring in a few days. The 2 cases presented the most protracted clinical courses noted in this series and at autopsy showed nodulation of the liver with histologic lesions indicative of recrudescence.

**CASE 9.**—The patient was a chief machinist's mate in the United States Navy, 28 years of age. The duration of his hepatitis was sixty-two days. He complained of weakness and fatigability for three weeks and of anorexia, jaundice, abdominal distress and clay-colored stools for two weeks.

For three weeks, beginning about Nov. 20, 1943, there had been a gradual onset of weakness and fatigability. One week after onset jaundice developed along with anorexia, epigastric distress and clay color of stools. The patient was admitted to the sick list while attached to his ship and was transferred to a United States naval hospital December 10, with the diagnosis of acute infectious jaundice.

His temperature was 99 F., pulse rate 80 and respiratory rate 20. There was a marked jaundice of the skin, the scleras and the mucous membranes. The abdomen revealed no abnormality except for tenderness along the left costal margin and in the midepigastrium.

The jaundice, which was marked, did not recede. The icterus index, which was 187 units on the day of entry, gradually reached 266 on Jan. 12, 1944. During this time his blood showed leukopenia, with leukocyte counts ranging from 5,400 to 6,450. Roentgenograms revealed an extrinsic duodenal shadow interpreted as representing a possible lesion of the pancreas. Ascites became apparent on January 12. Exploratory laparotomy was decided on. However, January 19, the day before he was scheduled for operation, a sudden epigastric pain developed and the abdomen became rigid. Emergency laparotomy was performed, local anesthesia being used, and 3,000 cc. of ascitic fluid was removed. Inspection of the liver revealed multiple dark nodules interpreted by the surgeon as "melanosarcomatous." The postoperative course was rapidly downhill, the patient becoming comatose and dying two days later, January 21.

The clinical diagnosis was metastatic melanosarcoma of the liver, with obstructive jaundice.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed marked icterus. Contained within the peritoneal cavity was 1,000 cc. of slightly turbid, blood-tinged fluid. Both layers of the peritoneum were markedly hyperemic and in places fiery red.

The liver was markedly flattened and flabby and weighed 860 Gm. Glisson's capsule was especially wrinkled over the main lobe. On cut section there was a contrast between the two lobes. The right lobe presented a mottled purplish

red to orange appearance, with absence of lobular markings, and the left lobe a homogeneous, pale, brownish tan appearance, with indistinct lobular markings. On the posterior aspect near the hilus, as well as in the caudate and quadrate lobes, there were indistinct greenish tan nodular areas which measured up to 1.5 cm. in width. These showed lobular markings. The surrounding liver tissue was flabby and brownish red. Several histologic sections showed complete absence of liver cells. Residual groups of small perilobular bile ducts were numerous. Tiny groups of lymphocytes were scattered throughout. Other sections showed faint outlines of the lobule with an attempt at liver cell regeneration. However, most of the liver cells were uniformly swollen, granular and devoid of nuclear staining.

The spleen was moderately enlarged, weighed 260 Gm. and measured 17 by 10 by 3 cm. Its pulp was soft and slightly opaque and presented a mottled brownish gray appearance. Histologically it showed marked hyperemia.

The heart had numerous recent friable vegetations on both leaflets of the mitral valve. There was but slight fibrous thickening of the leaflets. Histologic sections of the valve showed recent bacterial endocarditis, which was associated with extensive hemorrhagic extravasation beneath the adjacent endocardium.

The testes were rather small and microscopically showed marked arrest of spermatogenesis.

The anatomic diagnosis was: acute epidemic hepatitis with jaundice; mild arrest of spermatogenesis; acute bacterial endocarditis of the mitral valve.

CASE 10.—The patient was a chief radioman in the United States Navy, 23 years of age. The duration of his hepatitis was uncertain but was estimated to have been approximately forty-seven days. The complaint was generalized intermittent abdominal pain lasting ten days.

In November 1943 the patient had been admitted to another naval hospital with the diagnosis of gastritis. He was discharged to duty on December 8. Several days before Christmas, abdominal pain of an intermittent nature developed, which was generalized over the whole abdomen. On the day the patient entered the hospital, December 31, the pain had localized in the right lower quadrant of the abdomen, and the patient vomited twice.

His temperature was 102.4 F., pulse rate 68 and respiratory rate 24. He was well developed but appeared acutely ill and was rather stuporous. His abdomen was slightly distended and presented boardlike rigidity, with generalized tenderness, which was most marked in the right lower quadrant. Peristalsis was markedly diminished.

It was the opinion of the examining surgeons that he had generalized acute peritonitis secondary either to rupture of the appendix or to peptic ulcer. Three days later, the scleras became icteric, the abdomen remained distended, and his temperature fluctuated from 101 to 104 F. On January 4 the abdominal distention was less, and spasm of the abdominal muscles was absent. Severe anemia rapidly developed. The red blood cell count, which was 4,210,000 on December 31, dropped to 1,560,000 on January 4. The white cell count varied from 6,200 on December 31 to 9,100 on January 5. Transfusions of whole blood were given. On the following day, ascitic fluid was detected, and 500 cc. was removed. Two days later, January 7, the abdominal distention became more marked, rales developed at the base of each lung, and the patient died eight days after entering the hospital. His icterus index had varied from 50 to 111 units.

The clinical diagnosis was acute peritonitis and jaundice of uncertain origin, possibly secondary to perforation of acute appendicitis.

*Pathologic Observations.*—The skin, the scleras and the mucous membranes showed marked icterus. The right breast weighed 56 Gm. and showed moderate gynecomastia. Contained within the peritoneal cavity was 4,600 cc. of icteric ascitic fluid.

The liver weighed 1,890 Gm. Glisson's capsule was irregular in contour owing to coarse underlying nodulation. The parenchyma was divided into faintly perceptible nodules, which varied from 0.7 to 0.9 cm. in width. It was icteric and brownish orange. Lobular markings were indistinct. There was demonstrable increase in fibrous tissue on cut section. Microscopically, nodules of hyperplastic liver tissue were separated from one another by nonhyalinized fibrous stroma, which contained many groups of proliferating perilobular bile ducts. Small groups of lymphocytes were scattered throughout. Some of the liver cells were large, stained poorly and were detached from one another. In some areas necrosis of liver cells was extensive. In some sections it involved chiefly the central and midzonal portions of the lobules, although some lobules were completely involved.

The spleen weighed 585 Gm. and measured 20 by 12 by 5.5 cm. Its pulp was firm and reddish brown. Histologically, the pulp was markedly engorged with blood.

The testes showed moderate atrophy of the seminiferous tubules with subtotal arrest of spermatogenesis.

The anatomic diagnosis was as follows: acute epidemic hepatitis with areas of regeneration and secondary necrosis; ascites; moderate arrest of spermatogenesis; gynecomastia.

#### COMMENT

Four fairly distinct groups of cases are presented in this series of 10 fatal cases of epidemic hepatitis. In 2 cases mental and neurologic disturbances dominated the clinical picture. Such disturbances were present though not dominant in the terminal stages of nearly all the other cases (see table). Spontaneous rupture of the spleen with fatal hemoperitoneum occurred in 1 case on the second day of illness, affording an opportunity to study necropsy material at an unusually early stage of the disease. In 5 wounded men an unusually virulent or overwhelming type of hepatitis developed (third group of cases). In this group the possibility could not be excluded that the icterogenic agent had been introduced by transfusion of whole blood or plasma within an interval varying from three to three and a half months previously. Not only was the course uniformly much more rapid than usual, but the classic preicteric phase of symptoms was either absent or masked by conditions referable to the wounds which these patients had sustained. All had acute diffuse lesions of the liver, and again an opportunity was afforded of studying the tissue changes at earlier stages in this disease than has been possible with the more common, less overwhelming infections, which the patients survive considerably longer. The fourth group in this series consisted of 2 cases in which the clinical course and findings conformed more closely to those noted in other outbreaks of the disease. These 2 patients were the only ones who survived longer than ten days. Symptoms were referable primarily to the abdomen. In 1, in whom the

illness lasted about forty-seven days, hepatic lesions were revealed which were highly indicative of a recurrence superimposed on a considerable degree of regeneration. The liver of each patient showed nodulation in contrast to those of the other 8 patients in which the hepatic lesion was early, diffuse and free of nodulation.

An unusual feature of the clinical course in 8 cases was the brevity of the preicteric phase; either it was absent or it was masked by other conditions. Ordinarily, the course of fatal hepatitis falls into three phases: preicteric, intermediate and fatal. In the majority of cases the preicteric phase lasts seven days or less, although it may last as long as eighteen. Havens,<sup>23</sup> in reporting a series of 200 clinical cases in the Middle East, stated that 83.5 per cent showed a definite preicteric phase, which varied from one to eighteen days, with an average of five days. In the army series of 125 fatal cases reported by Lucke<sup>14a</sup> the preicteric phase varied from one to seventeen days, being in the majority seven days or less.

The intermediate phase in the present series of cases was likewise characterized by its brevity, lasting only seven days or less in the majority of instances. This is in contrast to representative data published elsewhere. For example, in Havens' series it varied from four to eighty-three days, with an average of twenty-seven days. In Lucke's<sup>14a</sup> series it varied from three to forty-three days and in the majority of cases it lasted twenty-six days or less.

The striking brevity of the clinical course in the present series of cases no doubt accounts for the fact that in 80 per cent nodulation of the liver was either absent or barely perceptible and for the fact that the histologic lesions were observed at a much earlier stage than has been generally seen heretofore. In a few of the cases a mottled pattern of the freshly cut surface characteristic of lobular outlines persisted. On gross inspection the chief findings at the early stage were flabbiness of the liver, variable slight wrinkling of Glisson's capsule and variable decrease in weight. In a majority of the cases the liver possessed the consistency of a wet rag but on cut section showed persistence of lobular markings (fig. 3A). The lesions, therefore, were diffuse. By contrast, only in cases 9 and 10, in which the clinical course was longest, was the liver nodular. Typically the clinical picture in the intermediate phase gives no indication as to whether or not the disease is going to run its usual benign course. In a majority of the cases the icteric phase was so short and the patients so ill that a clearcut transition between the intermediate and the final phase was not apparent. Ordinarily the final phase is ushered in by a sudden dramatic change for the worse. Lucke<sup>14a</sup> noted

that death usually occurs within ten days after the onset of the final phase, and in his series there was a spread varying from two to eighteen days.

*Pathologic Anatomy.*—(a) Liver: The size of the liver, the presence of ascites, the size of the spleen and the complications are shown in the table. In 50 per cent of the cases the liver was definitely shrunken, possessing a weight of 1,135 Gm. or less. In no instance did it weigh in excess of 1,890 Gm. Generally, normal weight of the liver was observed in cases in which death occurred in the earliest stages of the disease or in cases of longer duration in which regenerative hyperplasia had occurred. The most characteristic finding was flabbiness of the hepatic parenchyma, which was noted even though a mottled pattern of lobular markings frequently persisted. In the 2 cases in which survival was longest the parenchyma was nodular, owing in part to irregular distribution of the lesions within the liver and in part to attempted regeneration of liver cells. In these older cases was seen also the greatest degree of bile duct proliferation. Microscopically, in most of the cases there were classic changes, characterized by diffuse hepatocellular necrosis and autolysis, predominantly central and midzonal, variable degrees of endophlebitis involving the central venules and rapid removal of cellular debris. Infiltration with inflammatory cells in variable degrees, chiefly lymphocytes, was evident in all cases. In spite of hepatocellular necrosis there was retention of lobular structure and no scarring. Hepatocellular necrosis had not progressed to diffuse autolysis of liver cells in 2 cases. In case 3, in which death occurred in an extremely early stage, possibly on the second day of illness, only a few liver cells had autolyzed. There was, however, extensive alteration in the lobular structure. Most of the liver cells were large, swollen and detached from one another. Comparatively few liver cells, however, had disappeared. An inflammatory component characterized by infiltrating lymphocytes and mononuclear phagocytes was diffuse and more extensive in this case than in any of the others (fig. 1A). In case 7, in which death occurred on the sixth day, the hepatic lesion was slightly more advanced. Hepatocellular necrosis was extensive, but autolysis and débridement were incomplete. Ghostlike outlines and groups of swollen, granular, non-staining, dead liver cells were still plentiful in various sections (fig. 3B). In this case, too, the inflammatory component was striking but not as extensive as in case 3, in which death occurred at a still earlier stage. These 2 cases indicate that the intensity of the inflammatory component is transient, being most severe in the earlier stages. Apparently, the inflammatory changes subside somewhat after autolysis of liver cells has occurred and after the cell debris has been removed. Ordinarily, when sections of liver are seen in cases of hepatitis, autolysis is well advanced or has been completed. Uniquely, in these cases the early stages of destruction were seen, and it appears that swelling of the liver cells,

their detachment from one another and coagulative necrosis precede autolysis. The exact mechanism of the removal of debris is not understood, although because of its rapidity it is presumed to be enzymatic action.<sup>14a</sup> This series of cases would indicate that it is completed by the seventh and tenth days. Generally, many Kupffer cells, as well as mononuclear phagocytes, contained demonstrable but variable amounts of lipochrome pigment. An attempt at regeneration of liver cells was seen in only 2 cases (8 and 9). Proliferation of per lobular bile ducts, however, was observed in several. Ordinarily, in the vast majority of patients who recover, complete regenerative restitution of liver cells is thought to occur, although a sufficiently large number of cases has not been examined to determine this point with finality. The preservation of the reticular framework seems to provide a scaffolding for this reconstruction.<sup>14b</sup>

(b) Lymph Nodes: Acute lymphadenitis of a regional type was frequently notable. This was characterized not only by edema, lymphoid hyperplasia and infiltration of lymphocytes and mononuclear phagocytes but occasionally by occurrence of focal areas of necrosis. These areas involved chiefly germinal centers of lymphoid follicles (fig. 2D) and resembled similar necroses involving centers of malpighian bodies of the spleen.

(c) Spleen: In each instance the spleen was enlarged, the weight varying from 211 to 622 Gm. (table). Characteristically in the early stages it was not only enlarged but soft and boggy (cases 3, 6 and 8). Later it became firm and smaller, and still later, after a prolonged course, not only firm but enlarged again (case 10). Histologic studies showed in the early stages of the disease definite splenitis, characterized by the occasional occurrence of focal necrosis or of necrosis involving the central portions of large, hyperplastic malpighian bodies, lymphocytic infiltration of the recticulum of the pulp, and areas of edema in the supporting stroma (fig. 2A). These changes are variable in severity and occur only in the early stages of the disease. Later, the malpighian bodies become small and compact. The capsule becomes tense and friable in the early stages. Rupture of the splenic capsule occurred with resultant fatal hemoperitoneum in 1 case (3). In another case (8) the capsule was so tense and friable that it was readily lacerated at autopsy in spite of careful manipulation in its removal. Inasmuch as the lesions of splenitis are most marked in the early stages, before the process of cell death and autolysis observed in the liver has occurred or reached its acme, it seems probable that the splenic lesions are due to direct action of the icterogenic agent which is responsible for the characteristic lesions elsewhere (in the liver, the lymph nodes, the testes, and occasionally the heart, the lungs and the brain). To me this explanation seems more

likely than the hypothesis that "the early lymphoid hyperplasia is probably a reaction to products of tissue breakdown in the liver."<sup>11,12</sup> Splenic enlargement in the late stages after subsidence of the acute splenic enlargement seen in the first few days of the disease is most probably due to congestion and hyperplasia of the pulp.

(d) Testes: Even though variable degrees of arrest of spermatogenesis were noted in the majority of cases, both at early and at late stages, definite orchitis occurred at early stages in a number of cases (fig. 2B). The arrest of spermatogenesis may have been due to a number of factors —infection, fever, debilitation, metabolic disturbances subsequent to wounds, and nondisposal of estrogens concomitant with damage of the liver. Testicular changes characterized by arrest of spermatogenesis have been noted quite commonly but not universally by other writers, and it has been assumed to be due to a disturbance in the disposal of estrogens occasioned by the damage of the liver. Orchitis, on the other hand, does occur in the early stages, is representative of one of the systemic lesions of the initial disease and may well be an additional factor promoting early arrest of spermatogenesis.

(e) Kidneys: A variable, usually mild, type of cholemic nephrosis occurred in several cases.

(f) Brain: No characteristic anatomic lesions were found to account adequately for the neurologic disturbances, which were often fleeting and occasionally profound. In cases 1 and 2 neurologic and mental disturbances were profound, culminating in 1 case with the occurrence of decerebrate rigidity. The most common findings were in the mesencephalon, the brain and the cerebral white matter. These consisted of marked dilatation of the tiny blood vessels, some of which contained recent thrombi, and occasional small perivascular hemorrhages. Mild meningoencephalitis was found in 1 case. It seems clear that physiologic disturbances, coincident possibly with failure of an enzyme system concerned with carbohydrate metabolism, rather than morphologic, in cases of severe hepatic damage are responsible for the neurologic manifestations. One unusual coincidental intracranial finding is worthy of especial comment, namely, an area of internal hemorrhagic pachymeningitis with scarring of the underlying cerebral cortex in a patient who had sustained an immersion blast injury four months previously. Recent bleeding may well have been accentuated by an acute hemorrhagic diathesis consequent to damage of the liver and impaired formation of prothrombin. The intracranial lesion is of great coincidental interest from the point of view of etiology. In the absence of syphilis, alcoholism, recent trauma and other factors commonly associated with occurrence of such a lesion there is the definite suggestion that it may have occurred as the sequel to immersion blast injury. To date there have been reported no instances

of dural and adjacent cortical lesions developing secondary to such a trauma.

(g) Gastrointestinal Tract: Phlegmonous inflammation and edema of the gastrointestinal tract were not noted. Such phenomena have been reported when the patient survived for a much longer interval.<sup>14a</sup> Their absence in the cases of the present series, in most of which death occurred early, would indicate that phlegmonous inflammation and edema are sequelae. No gastric lesions were observed such as have been recently alluded to by Knight and Cogswell.<sup>24</sup>

(h) Hemorrhagic Phenomena: Of variable degree and occurrence were the small ecchymotic hemorrhages in the epicardium and the mucosa of the renal pelvis and beneath the endocardium of the left ventricle (septum and papillary muscles). As previously stated, it is thought that decreased formation of prothrombin secondary to severe damage of the liver is most probably responsible for their occurrence.

(i) Lungs: In 2 cases interstitial pneumonitis was shown.

(j) Heart: In case 3 there occurred definite myocarditis, which was characterized by infiltrating lymphocytes and mononuclear phagocytes. These were most numerous beneath the endocardium and in the papillary muscles.

#### SUMMARY

Fatal acute epidemic hepatitis occurred in 10 members of the naval personnel. The age of these patients varied from 19 to 28 years.

Six of the patients who died had been wounded three to four months previously. In this group of previously wounded men the possibility of "homologous serum jaundice" with maximum periods of incubation up to four months could not be excluded. The hepatitis in this group was fulminating and characterized by a clinical course of ten days or less. The classic preicteric phase usually seen in acute epidemic hepatitis was either absent or obscured by other conditions. In 1 case the cephalin cholesterol flocculation test gave a positive result on two occasions one month before the advent of clinical jaundice. The mortality rate in this group was 19 per cent in contrast to the characteristically low mortality rate (0.13 to 0.44 per cent) reported in the literature for cases of epidemic hepatitis.

Inasmuch as 80 per cent of the total number of patients died within from two to ten days after the onset of hepatitis, it was possible to study the lesions at an early stage in their development. This was a much earlier stage than in other outbreaks, in which the disease ran a more protracted course.

24. Knight, W. A., and Cogswell, R. C.: J. A. M. A. **128**:803, 1945.

One patient died on the second day of illness from a spontaneous rupture of the spleen with an ensuing fatal hemoperitoneum.

Consistently, the livers showed changes similar to those of "acute yellow atrophy." At the earliest stage at which the lesions were examined (second day), the liver cells were swollen and detached from one another, and only minimal autolysis had occurred. By the tenth day, however, most of the damaged liver cells had become autolyzed, and the cellular debris had been removed. An accompanying inflammatory component, consisting chiefly of the presence of lymphocytes and monocytes, was most marked in the earlier stages. Gross nodulation was either absent or minimal in the livers of patients who lived ten days or less.

The spleen showed varying degrees of enlargement. In a number of instances necrosis was observed in the germinal centers of the splenic malpighian bodies and regional lymph nodes. Characteristically, the regional lymph nodes were enlarged and spongy and showed lymphadenitis.

Uniformly, arrest of spermatogenesis occurred, which in a majority of the cases in which the lesions were in an early stage was associated with definite orchitis.

Miscellaneous lesions occurred in several cases in which the disease was studied at an early stage. Interstitial pneumonitis was present in 2 instances. A mild type of meningoencephalitis was found in 1 instance and myocarditis in 1 instance.

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# CHEMICAL FACTORS AND THEIR ROLE IN INFLAMMATION

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CELLULAR injury involves a radical alteration in the biochemistry of the cell. The consequence is the liberation of various common denominators, which in turn are readily recovered from the exudative fluid bathing the injured cells. The presence of these substances is of aid in explaining the basic pattern of numerous processes that accompany severe injury. Inflammation is essentially a manifestation of severe cellular injury in the tissues of higher animals. The phenomenon of inflammation requires the presence of vascular channels, lymphatic structures and tissue cells besides the emigrating leukocytes and the outpouring of fluid from the circulating blood.

In connection with studies of exudates a crystalline-like nitrogenous substance was isolated. This material is per se capable of explaining reasonably the increased capillary permeability and the migration of leukocytes in acute inflammation. It has no apparent relation to histamine. To it the name "leukotaxine" has been assigned.<sup>1</sup> Another type of material liberated by the injured cells in inflammation is glucose. The surplus sugar produced, probably by deamination of the protein molecule at the site of injury, offers an explanation of the enhancement of diabetic conditions that frequently presents itself with infections.<sup>2</sup> Fundamentally, any injured cell is potentially gluconeogenetic.<sup>3</sup>

I shall not comment further on either leukotaxine or the glucose produced at the site of acute inflammation. Rather I shall devote the rest of this paper to more recent studies on the leukocytosis-promoting factor, on the pattern of injury (necrosin), on the pyrogenic factor (pyrexin) and on a more recently studied leukopenic factor which appears at the site of acute inflammation.<sup>4</sup>

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From the Department of Pathology, Duke University School of Medicine.

The Gamma Chapter of Sigma Zeta Lecture delivered before the Medical College of Virginia, Richmond, May 15, 1945, as revised for an address at the University of Chicago, Dec. 7, 1945.

1. Menkin, V.: Dynamics of Inflammation, New York, The Macmillan Company, 1940.
2. Menkin, V.: Am. J. Physiol. **134**:517, 1941.
3. Menkin, V.: Am. J. Physiol. **138**:396, 1943.
4. Menkin, V.: (a) Am. J. Path. **16**:13, 1940; (b) Arch. Path. **30**:363, 1940.  
(c) Menkin, V., and Kadish, M. A.: ibid. **33**:193, 1942. (d) Menkin, V.: Am. J. Path. **19**:1021, 1943; (e) Arch. Path. **36**:269, 1943; (f) Am. J. M. Sc. **208**:290, 1944; (g) Arch. Path. **39**:28, 1945; (h) **41**:50, 1946.

## THE MECHANISM OF LEUKOCYTOSIS WITH INFLAMMATION

Leukotaxine is concerned with the leukocytes migrating locally into an area of acute injury, but it seems to have little to do with regulating the number of circulating white cells.<sup>4a</sup> When introduced intravenously into a rabbit for several successive days it fails to alter appreciably the leukocytic level in the blood stream. This is true likewise in the dog. On the other hand, the absolute number of leukocytes can be readily increased within several hours by injecting into the circulation whole or cell-free exudative material. This observation suggests that exudate contains a leukocytosis-promoting factor, particularly if it is derived from a dog with pleural inflammation and concomitant circulatory leukocytosis. No such rapid effect can be elicited with normal canine blood serum or with various other materials, such as isotonic solution of sodium chloride, broth or even bacteria.

The leukocytosis-promoting factor (abbreviated in the accompanying charts to LPF) is thermolabile, being inactivated at 60 C. It is nondiffusible. These facts have suggested that it is of a protein nature. Fractionation by ammonium sulfate indicates that the active principle is primarily located in the pseudoglobulin fraction, i. e., in the fraction precipitated at one-half saturation with ammonium sulfate.<sup>5</sup> Preliminary studies in collaboration with Dr. Gerald Cooper with the Tiselius cataphoretic apparatus indicate that the leukocytosis-promoting factor seems to be associated with the  $\alpha_2$ -globulins of exudates. The active principle is not present in normal serum, but it may be recovered from the blood serum of an animal with a concomitant acute inflammation.<sup>4c</sup> The fact that this factor is liberated into an inflamed area and subsequently penetrates into the blood stream offers a reasonable explanation of the basic mechanism of the leukocytosis that frequently accompanies inflammatory processes. These observations have been made on dogs and on rabbits. Reifenstein and his collaborators have substantiated these studies of exudative material on rabbits.<sup>6</sup> Recently Mattison and I, in as yet unpublished studies, succeeded in obtaining

5. With further purification it has been found essential first to remove the euglobulin fraction of exudate in order to avoid a preliminary leukopenic phase. The final material is then dried by freezing. In this state the leukocytosis-promoting factor soon tends to lose its potency. I have recently found that the material maintains relative potency in vacuo under phosphoric anhydride.<sup>3</sup> Nevertheless, even by this procedure the activity of the material is not sustained for too long. It has been found that activity can be prolonged by maintaining the active material in the presence of ammonium sulfate on ice. The sulfate ions are dialyzed out prior to use of the material. In this way the leukocytosis-promoting factor can be maintained for weeks in the fluid state on ice. After dialysis, drying by freezing can be employed.

6. Reifenstein, G. H.; Ferguson, J. H., and Weiskotten, H. G.: Am. J. Path. 17:233, 1941.

similar results in guinea pigs (fig. 1). This may render the latter a convenient test species in which to assay various fractions of the leukocytosis-promoting factor. These studies are being pursued further.

Has the leukocytosis-promoting factor any effect on the growth of cells in the bone marrow? The observations indicate that one to two days after it has been injected intravascularly into a dog, marked hyperplasia occurs in the marrow. The tissue becomes replaced with actively growing granulocytic elements and also with megakaryocytes.<sup>4d</sup> The findings warrant the conclusion that the leukocytosis-promoting factor favors hyperplasia both of granulocytes and of megakaryocytes in the marrow, presumably with immature polymorphonuclear leukocytes being simultaneously discharged into the circulation, causing

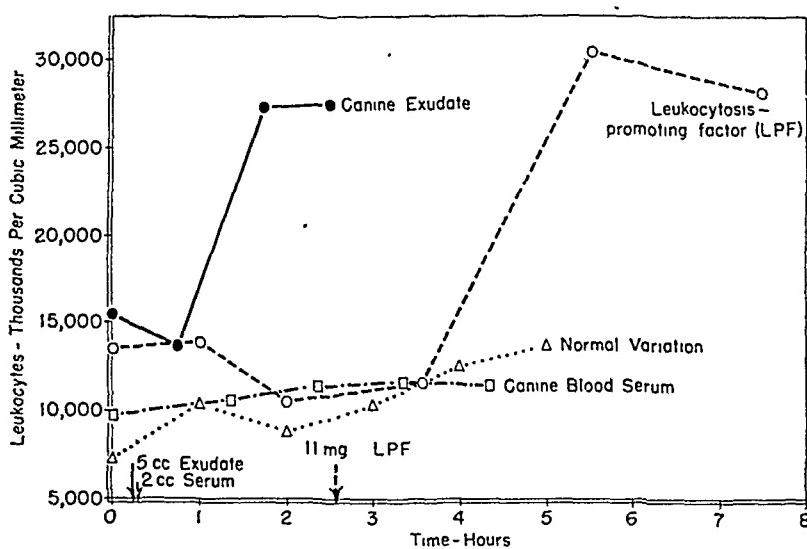


Fig. 1.—Effect of the leukocytosis-promoting factor on the number of the circulating leukocytes of the guinea pig. Canine exudative material when introduced into the peritoneal cavity of the guinea pig induces a sharp increase in the white cells. Identical results have been obtained with 11 mg. of the leukocytosis-promoting factor (LPF). No appreciable effect is observed after the introduction of blood serum. The leukocytosis-promoting factor can likewise be injected subcutaneously.

thus the leukocytosis that frequently accompanies inflammation. The clinical implications of such studies are obvious. The leukocytosis-promoting factor (canine) has been successfully injected intravenously into 10 human subjects. The material is both innocuous and active in amounts varying from 18 to 231 mg. This fact opens definite, and probably clinical, approaches to the general problem (figs. 2 and 3). In preliminary studies, to be reported later, it has been found that the leukocytosis-promoting factor seems to raise the white cell count of a leukopenic patient. One injection of 77 mm. of the material has raised the count from about 2,000 cells per cubic millimeter to over 4,000. The



Fig. 2.—Effect of the leukocytosis-promoting factor derived from canine exudative material on the number of circulating leukocytes of a human being. The subject, a Negro 41 years of age, received intravenously 127 mg. of the leukocytosis-promoting factor. (An arrow indicates the time of injection.) This promptly produced a rise in the number of circulating leukocytes (—). The rise was largely due to augmentation of the polymorphonuclear leukocytes (---), which is reflected by an increase in the number of immature neutrophils (one lobe, immature leukocytes). This clinical study is being conducted in collaboration with Dr. E. Ullid and Dr. E. G. Goodman.

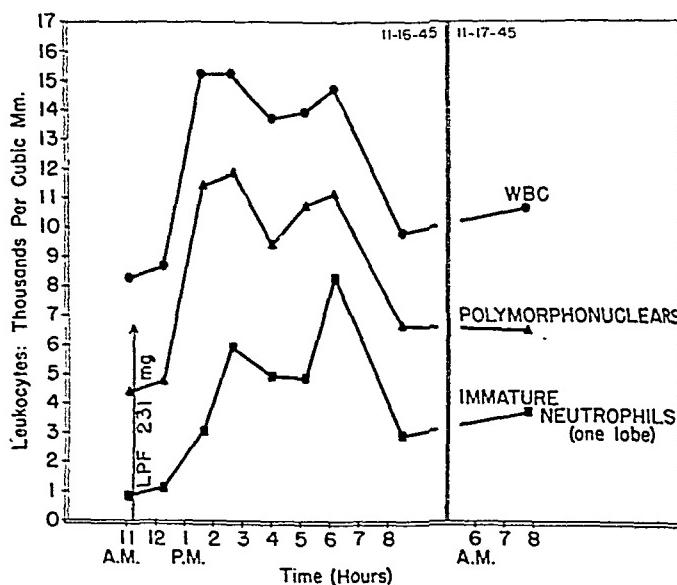


Fig. 3.—Effect of the injection of 231 mg. of the leukocytosis-promoting factor on the circulating leukocytes of a youth aged 19. Note the rapid rise in the number of white cells. This is primarily due to immature polymorphonuclear leukocytes discharged into the circulation.

new level has been sustained for at least a week. These studies are being continued.

#### THE BASIC MECHANISM OF INJURY IN INFLAMMATION

Close scrutiny reveals a fairly well delineated pattern in the development of an inflammatory reaction. This, for instance, involves the four fundamental cardinal signs described originally by Celsus, as well as a loss of function, as pointed out later by John Hunter. To these basic features one may add a biochemical change in the normal protein metabolism of the cell, namely, an increase in proteolysis or a rise in the products of protein breakdown. The fundamental pattern, as just outlined, may be altered depending on the chemical nature of the irritant and on the interrelationship of the latter with the particular tissue of the host. The precise anatomic location of the affected part may also be a factor to be considered. But by and large a basic reaction of injury persists throughout the course of inflammation.

Is there any conditioning factor to account for the pattern of injury in acute inflammation? The exudate per se when injected into normal tissue will induce an edematous reaction sufficiently intense to be accompanied by lymphatic blockade, an evidence of severe injury.<sup>7</sup> When the exudative material is analyzed, it has been observed that only the euglobulin fraction can elicit a marked inflammatory reaction in the skin of a rabbit.<sup>4e</sup> Further purification has revealed that an injurious factor is either located in the euglobulin fraction of exudate or else closely associated with that particular protein fraction, for neither the pseudoglobulin nor the albumin fraction of exudate induces any appreciable injurious effect.

This euglobulin fraction has of late been dissociated from a pyrogenic and a leukopenic factor, each of which had originally been found in close association with the euglobulin fraction of exudate.<sup>4e</sup> This injurious factor which per se reproduces the severe reaction of an acute inflammation has been termed necrosin.<sup>4e</sup> In general, though not always, it has been found that necrosin tends to be recovered more readily if the exudate from which the material is extracted is at an acid  $p_H$ . Originally it had been shown that with the progress in intensity of an acute inflammation a rise in the local hydrogen ion concentration occurs.<sup>8</sup> The local acidosis seemed primarily referable to a glycolytic process causing lactic acid acidosis. With the rise in acidity polymorphonuclear leukocytes are markedly injured, leaving the macrophages essentially unimpaired. With further progress in the reaction and a corresponding fall in the  $p_H$  to 6.5 or even below, all types of leukocytes are found injured, and a state of suppuration ensues. Pus formation is virtually a function of the hydrogen ion concentration.

7. Menkin, V.: Physiol. Rev. 18:366, 1938.

8. Menkin, V., and Warner, C. R.: Am. J. Path. 13:25, 1937.

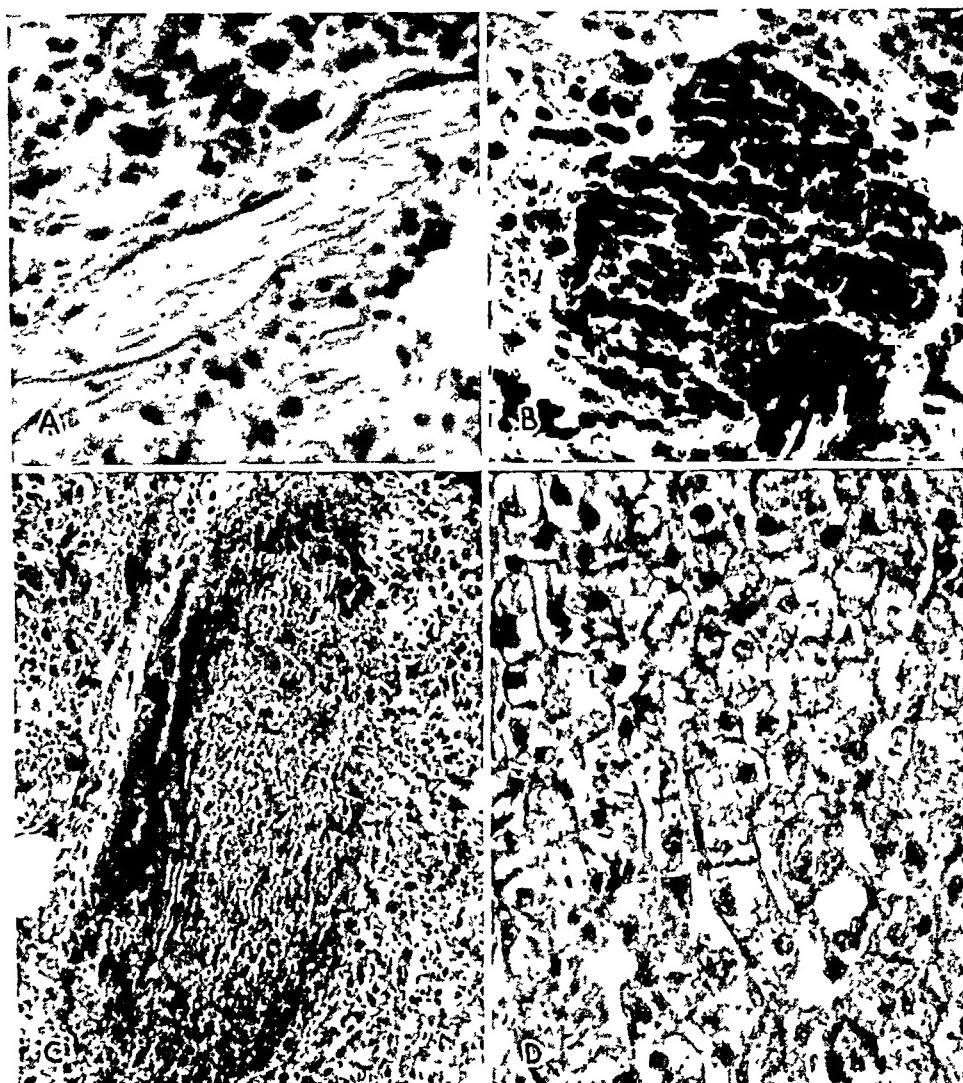


Fig. 4.—*A*, acute inflammatory reaction observed one day after necrosin had been injected into the skin of the abdomen of a rabbit. Note the occlusion of a lymphatic vessel by fibrinous plugs. This is evidence of the severe injury induced by necrosin.  $\times 445$ .

*B*, marked reaction evoked within seventeen hours after 0.5 cc. of a suspension of necrosin was injected into the abdominal skin of a rabbit. Microscopic examination revealed occlusion of lymphatic vessels, as shown in *A*.  $\times 485$ .

*C*, severe local inflammation induced by 0.5 cc. of a necrosin preparation injected into the skin of a rabbit. Seventeen hours after the injection the area was excised, and subsequent microscopic examination revealed not only occluded lymphatics but blood vessels showing strands of fibrin originating apparently from the endothelial lining. This probably can be considered to be an initial stage of the formation of a thrombus.  $\times 225$ .

*D*, denudation of cytoplasm of liver cells. Dog 3D received six intravenous injections of necrosin within a period of three weeks. The animal was then killed. The cytoplasm of the individual liver cell, as shown, seems to be denuded of its content. This appearance of the liver has been found to be referable to abundant deposition of glycogen.  $\times 485$ .

It is therefore not wholly surprising to find a tendency toward an increase in necrosin with the rise in hydrogen ion concentration in an acutely inflamed area. In this way it is conceivable that the greater formation of necrosin may further the reaction of injury with the increase in acidity.

The degree of injury in inflammation may be conveniently gaged by the development of lymphatic blockade. The lymphatic vessels become plugged with fibrin. Necrosin characteristically induces such a state of affairs (fig. 4 A and B). The pseudoglobulin and albumin fractions when introduced into tissue leave the lymphatic structures unaltered. In a tissue area in which necrosin has been injected, small vascular channels are found with damage of the endothelial lining (fig. 4 C). One of the earliest manifestations of the effect of necrosin injected into the abdominal skin of a rabbit is swelling of the collagenous bundles.

Necrosin is not present in normal blood serum, but it may be recovered to some extent from the serum of an animal with a concomitant acute inflammation. This fact suggests that necrosin is absorbed from the site of an acute inflammation into the circulating blood. Perhaps the fact that necrosin is present in the blood stream of animals with acute inflammation may be of significance in reorienting some present day notions concerning the role of foci of infection in lesions of organs situated at a distance. For this reason, studies have been undertaken to determine the effect of necrosin injected directly into the circulation.

#### THE EFFECT OF NECROSIN INJECTED INTO THE CIRCULATION

When injected into the circulation of a dog, a single dose of a suspension of necrosin induces most frequently some sort of hepatic injury. This may be manifested in the gross specimen by a tasselled appearance of whitish areas or streaks, which vary considerably in extent. Microscopic examination may reveal scattered foci of disintegrated cells containing a curious granular stippling, which fails to take the iron stain. It is conceivable that this represents nuclear debris. Sometimes the areas of injury reveal vacuolated cells with interspersed foci of leukocytic infiltration. More recently studies have been undertaken to determine what effect necrosin may have on the liver when repeatedly injected into the circulation. Here again injury or alteration of the organ seems to be a frequent feature, although the character of the change seems to vary from animal to animal. Whether this depends wholly on dosage and on the number of injections is not as yet settled. For instance, following two injections of necrosin considerable fatty degeneration has been encountered throughout the parenchyma. On the other hand, six intravascular injections of the material made into

a dog over a period of three weeks induced a curious evacuation of the cytoplasmic constituents in the liver, leaving a bare framework (fig. 4*D*). The empty spaces have sometimes failed to take the fat stain, but the glycogen present within the cells is so abundant as to leave little doubt that the microscopic appearance of the tissue seems primarily referable to large amounts of glycogen. The animals were starved for about one day prior to being killed, rendering it doubtful that the presence of enormous deposits of glycogen is referable to ingestion of large amounts of carbohydrates. Furthermore, control animals have failed to reveal any such deposits. Finally, when necrosin is administered intravascularly for longer intervals—for example, about two months—not only does there seem to be less deposition of glycogen but the latter appears to be heterogeneously distributed throughout the organ. The failure of the material to be homogeneously deposited in the liver cord suggests a mechanism other than mere ingestion of carbohydrate. It is perhaps conceivable that the formation of glycogen is referable in part to deamination of protein as a result of extensive hepatic cellular injury. These studies are being pursued further.

The kidney at times shows, following a single injection of necrosin, moderate vacuolation of the lining tubular cells, as well as foci of interspersed leukocytic infiltration. Following numerous injections of the material, a peculiar colloid-like material has been found within the capsular space of some of the glomeruli. The liver and quite often the kidneys are the organs most frequently involved after injections of necrosin. Occasionally, though not constantly, a condition of bilateral hydrothorax has been encountered, as well as small hemorrhages throughout the length of the gastrointestinal tract.

In brief, these studies, which are still in progress, at least suggest the significance of a toxic material elaborated by injured cells at the site of an acute inflammation. The absorption of such a substance may have definite repercussions on some of the visceral organs, notably the liver. These facts have to be borne in mind in the further study of the organism as a whole when one of its parts is undergoing a severe inflammatory process.

Smith and Smith<sup>9</sup> have recently described a toxic material in the euglobulin fraction of menstrual blood. Not only have they confirmed the presence of necrosin in exudative material but they also have advanced evidence indicating that necrosin and the toxic material in menstrual blood are identical in nature. This fact is not wholly surprising, for menstrual blood contains, besides blood, debris of cellular injury derived from the damaged endometrium.

9. Smith, O. W., and Smith, G. U. S.: Proc. Soc. Exper. Biol. & Med. 59:116, 1945.

Earlier studies indicated that necrosin manifests proteolytic activity. These studies have been substantiated further by Dr. Frederick Bernheim and me. These observations will be reported in detail elsewhere.

#### THE MECHANISM OF FEVER WITH INFLAMMATION

In the early studies on the necrosin present in inflammatory exudate, it had been observed that the euglobulin fraction of exudate, besides inducing local cutaneous injury, caused when injected intravascularly into dogs<sup>4e</sup> a concomitant rise of temperature and transitory leukopenia. These findings suggested further studies to determine whether the fever was referable to the necrosin or to some other substance present in exudate.

Under normal circumstances the dog's temperature hardly fluctuates during a period of about six hours. This is also seen when either the albumin of the pseudoglobulin fraction of exudate (leukocytosis-promoting factor) is introduced into the circulation. The euglobulin fraction of normal blood serum is also ineffective as far as eliciting a rise in temperature is concerned.<sup>10</sup> On the other hand, when the whole euglobulin fraction of exudative material is injected intravascularly a rapid rise in temperature occurs which in the series studied averaged about 3 F.<sup>10</sup>

These studies have been repeated on the rabbit as a convenient test animal.<sup>11</sup> A rabbit's temperature during a period of about six hours fluctuates maximally on the average about 0.6 F. unless there is extreme hot weather, in which the temperature of the animal may rise about 1 F. Saline solution, the euglobulin fraction of normal canine or human blood serum and the leukocytosis-promoting factor of exudate are all incapable of inducing an appreciable rise in body temperature. When the exudate obtained from the pleural cavity of a dog into which turpentine was previously injected is introduced into the circulation of a rabbit, a rapid rise in the animal's temperature frequently occurs. The average increase is about 2.3 F. This significant rise is duplicated only when the euglobulin fraction of the exudate is used, the average increase being about 2.5 F. Neither blood serum nor its euglobulin fraction evokes any appreciable fever; at least the fever does not rise to the same level as that elicited by the euglobulin fraction of exudate.

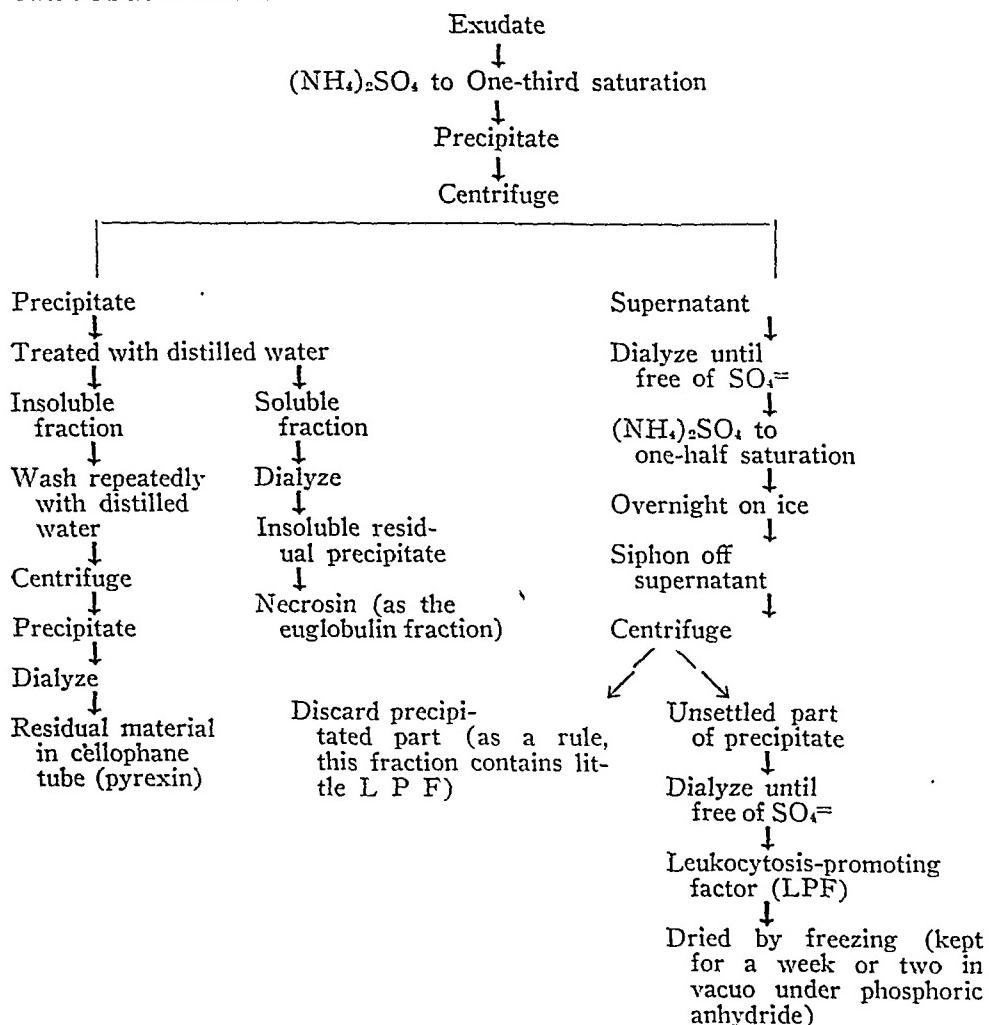
In the early studies it was noted that the euglobulin fraction maintained itself as a suspension in the presence of electrolytes. This seemed to be contrary to the accepted notion of euglobulins. It appeared at first as if one were dealing with an atypical euglobulin.<sup>11</sup>

Further studies, however, soon showed that one was really dealing with two different substances. One of these was the euglobulin fraction

10. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **54**:184, 1943.

11. Menkin, V.: Federation Proc. **3**:32, 1944.

proper which proved toxic when injected into the skin of a rabbit. This was necrosin. Necrosin thus far has failed to be dissociated from the true euglobulin fraction of exudate. But in the same fraction there exists another fraction incapable of entering into solution in the presence of electrolytes. This fraction, which can often be dissociated by differential solubility from necrosin or the euglobulin fraction of exudate, tends to be extremely pyrogenic in contrast to purified necrosin. The latter is scarcely capable of inducing fever. In brief, the scheme of the recovery of necrosin and the fever factor, termed pyrexin, and the modified method by which the leukocytosis-promoting factor may be extracted from a sample of exudate can perhaps be conveniently outlined as follows:



The fact that pyrexin is often, though not always, recovered from exudates that are at an acid  $pH$  to start suggests an explanation for the primary mechanism of the fever that occurs with inflammation. There is some evidence that this fraction acts on the heat-regulating

center in the hypothalamic region, for pentobarbital sodium and anti-pyretic drugs inhibit the full biologic activity of this substance. The latter substances are supposed to inhibit the action of the heat-regulating centers in the central nervous system. There is also some evidence that pyrexin is eliminated at least in part in the urine. A dog with an acute pleural inflammation gradually excretes this pyrogenic factor in the urine.<sup>4g</sup>

Pyrexin is heat stable. Boiling fails to inactivate it. When tested with ninhydrin, the reaction is positive. In the biuret test the reaction is positive but only in minute trace. Contrary to earlier observations, further studies have indicated that it gives a positive reaction in the Molisch test. Observations made in collaboration with Dr. Frederick Bernheim, which will be reported separately, indicate that pyrexin may be a relatively simple polypeptide. In view of the constant presence of a carbohydrate grouping (giving a positive reaction in the Molisch test), it seems as if pyrexin may be a glycopeptide.

Studies have been made of the mechanism of leukopenia, which frequently occurs with some inflammatory processes.<sup>4h</sup> There is evidence that a leukopenic factor closely but apparently not invariably associated with pyrexin is liberated in exudate. The nature of this factor seems to be that of a polypeptide. The leukopenia appears to be referable to a trapping of leukocytes in the lungs, the liver and the spleen. The white cells that accumulate in the spleen during the leukopenic phase may prove of significance in the further understanding of the mechanism of the acute splenic tumor with inflammatory processes. This is being studied. In general it is possible that the ultimate picture in the organism with an acute inflammation is at least to some extent the resultant of the amount of leukocytosis-promoting factor, of necrosin, of pyrexin and of leukopenic factor liberated at the site of an acute injury. I hope that sufficient material has been brought forward in these pages to indicate the significance of studying further the chemistry of injured cells. Thus, through an understanding of the nature of various liberated common denominators, one could perhaps attain a clearer concept of the basic mechanisms concerned in inflammation, which is the physical basis of infectious diseases in the host.

#### SUMMARY AND CONCLUSIONS

The development of the inflammatory reaction follows a fundamental pattern. This in turn is conditioned by some common denominators that can be extracted from the exudative material. For instance, the increase of capillary permeability and the migration of polymorphonuclear leukocytes are primarily referable to leukotaxine, presumably from injured cells. Glucose is likewise produced by damaged cells.

The leukocytosis frequently accompanying inflammatory processes is apparently due to a protein in the pseudoglobulin fraction of exudate. This substance, besides inducing a discharge of immature granulocytes from the bone marrow, also causes a marked hyperplastic reaction of these elements and of megakaryocytes in the marrow. The leukocytosis-promoting factor (LPF) affects the circulating leukocytes of dogs and of guinea pigs. The canine leukocytosis-promoting factor is both innocuous and active in human beings. When injected into the blood of man it induces a rise in circulating leukocytes ranging from 80 to 150 per cent.

The pattern of injury causing inflammation seems primarily referable to a toxic euglobulin liberated by injured cells. This factor has been termed necrosin. When intravascularly injected it induces injury of the liver and frequently of the kidney. The injury of the liver varies from a denudation of the cytoplasm of the hepatic cell and replacement with abundant glycogen to a large deposition of fat.

The fever that occurs with inflammation seems referable to what is possibly a glycopeptide, termed pyrexin. Pyrexin appears to act on the heat-regulating centers of the central nervous system. Although purified necrosin is nonpyrogenic, incubation of this substance frequently yields a pyrogenic substance, suggesting that pyrexin is possibly the product of enzymatic activity associated with necrosin.

The leukopenia often observed with inflammation seems referable, at least in numerous instances, to a leukopenic factor which can be recovered from exudative material, particularly if the latter is at an acid  $p_H$ . The leukopenic factor is often in close association with pyrexin, and it appears to be a heat-stable polypeptide. Its action appears to be a trapping of leukocytes in the alveolar walls of the lung, in the sinusoids of the liver and in the spleen. The cells retained in the spleen may perhaps be of aid in the further understanding of the acute splenic tumor accompanying numerous inflammatory processes.

## ADENOCARCINOMA OF THE URACHUS INVOLVING THE URINARY BLADDER

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THE paucity of reports of adenocarcinoma of the bladder arising from the urachus is rather striking, since neither tumors of the bladder nor urachus rests are particularly rare. Cases of tumor of the bladder constitute 3 per cent of all urologic cases (Verhoogen<sup>1</sup>), a figure which is in close agreement with the 4 per cent determined by Young<sup>2</sup> in an analysis of 12,500 cases.

In regard to urachus rests, Wutz<sup>3</sup> found 24 cysts in 74 cases, while Morse<sup>4</sup> was able to determine the existence of 13 cysts or patent urachal tubules in 21 consecutive autopsies. Begg<sup>5</sup> stated that careful dissection and histologic search would reveal urachal rests or cysts in the majority of the cases examined, a fact which appears to be substantiated by Saphir and Kurland,<sup>6</sup> who demonstrated the presence of typical tubular structures in the vault of the bladder in the region of the ligamentum umbilicale mediale in 9 of 10 bladders.

Notwithstanding this frequency, Begg<sup>5</sup> in 1931 succeeded in collecting only 19 cases of colloid adenocarcinoma from among 29 assorted cases of urachal tumor involving the bladder wall and the space immediately above it. This total did not include the case of Lane and Morson,<sup>7</sup> which appeared in 1930. Begg<sup>8</sup> added an additional case in 1936,

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1. Verhoogen, J., in Pousson, A., and Desmons, E.: *Encyclopédie française d'urologie*, Paris, G. Doin, 1921, vol. 4, p. 225.

2. Young, H.: *Practice of Urology*, Philadelphia, W. B. Saunders Company, 1926.

3. Wutz, J. B.: *Virchows Arch. f. path. Anat.* **92**:387, 1883; cited by Rankin and Parker.<sup>12</sup>

4. Morse, H., cited by Rankin and Parker.<sup>12</sup>

5. Begg, R. C.: *Brit. J. Surg.* **18**:422, 1931.

6. Saphir, O., and Kurland, S. K.: *Urol. & Cutan. Rev.* **43**:709, 1939.

7. Lane, C. R., and Morson, A. C.: *Brit. J. Urol.* **2**:271, 1930.

8. Begg, R. C.: *Brit. J. Surg.* **23**:769, 1936.

while in the same year Ferrier, Craig and Foord<sup>9</sup> reported 2 cases. Saphir and Kurland increased the total to 24 by publishing 1 case in 1939. Ash<sup>10</sup> found only 2 cases of adenocarcinoma which he thought might be derived from urachus rests, out of a total of 2,000 cases of tumor of the bladder which were registered at the Army Medical Museum.

Although urachal tumors are rare, it is important that each one that occurs should be differentiated from other tumors that may occur in the vault of the bladder, since the accepted therapy of papillary tumors, such as fulguration or radium implantation, is valueless, whereas surgical removal of the young lesion offers some hope.<sup>11</sup> The fundamental study of urachal tumors has been furnished by Begg,<sup>5</sup> from whose article the following details are abstracted, since his paper appeared in an otherwise not readily accessible journal.

#### ORIGIN AND DEVELOPMENT OF THE URACHUS

The derivation and the development of the urachus are described in most standard embryologic texts, to which the reader is directed. For the purpose of the present paper, the following brief description will serve to orient the subject.

The primitive hindgut in the early fetus is the anlage of the rectum, the upper part of the bladder and the urachus. This cavity, the cloaca, is lined by a single layer of cuboidal cells. With the formation of the urorectal septum, the cloaca becomes divided into the bladder and the urachus on one side and the rectum on the other. The cuboidal epithelium accordingly undergoes differentiation into the transitional epithelium of the bladder and the cylindric intestinal epithelium of the rectum. The epithelium of the urachus, however, belonging to an organ of no particular function, undergoes no specialization, in contrast to that of the rectum and the bladder, but remains as a primitive layer retaining all of the potentialities of primitive cells. This retention of potency is the key to the understanding of the various types of tumor of the urachus. Complete but ill regulated development toward glandular or transitional epithelium may overstep all bounds and give rise to colloid adenocarcinoma, on the one hand, or squamous cell epithelioma, on the other. Both types were described in a single case by Rankin and Parker<sup>12</sup>; usually,

9. Ferrier, P. A.; Craig, L. G., and Foord, A. G.: Urol. & Cutan. Rev. **40**: 457, 1936.

10. Ash, J. E., cited by Fleischman, A. G., and Mauritz, E. L.: J. Urol. **47**:658, 1942.

11. Lowsley, O. S., and Kirwin, T. J.: Clinical Urology, Baltimore, Williams & Wilkins Company, 1944, vol. 2, p. 1059.

12. Rankin, F. W., and Parker, B.: Surg., Gynec. & Obst. **42**:19, 1926.

however, the endodermal impulse predominates, with the resulting formation of adenoma and eventually of adenocarcinoma.

#### CLASSIFICATION OF URACHAL TUMORS AND PATHOLOGIC ANATOMY OF THE URACHUS

The urachus measures 5 to 6 cm. in length and may be subdivided into various sections: (1) supravesical where it is entirely above the bladder; (2) intramural where it lies within the muscle wall, and (3) intramucosal where its lumen is in direct continuity with the lumen of the bladder; this continuity occurs in about 33 per cent of the cases. The centimeter which penetrates the wall is the most important from the pathologic point of view, for it is usually at the upper part of this portion or in the lower end of the extravesical section that new growth originates. On the basis of the foregoing subdivision the tumors may be classified as intramucosal, intramural or supravesical.

Most of the specimens described fall into one of two varieties. The tumors of the first group possess typical characteristics. The upper part is encapsulated and extends well into the space of Retzius, while the lower end, which is in the bladder wall, has no definite capsule, the acini lying in direct contact with the muscular tissue. The majority penetrate the mucosa, but the length of the symptomless urinary history, combined with their large size and the presence of calcification in the capsule and the stroma, lead to the conclusion that they exist for a considerable length of time as simple tumors and only late in their course take on malignant characteristics.

The neoplasms of the second group apparently arise from adenomatous structures of the urachus and become cancerous at an early stage. This type is almost entirely within the wall of the bladder, and there is no capsule. The tumor is usually found by cystoscopy or at postmortem examination, and masses are usually not palpable.

In the first group, to which our specimen belongs, the growths range in size from that of a hazelnut (Kielleuthner<sup>13</sup>) to that of an adult head (Pendl<sup>14</sup>). There are usually two components, a supravesical cystic and an intramural solid portion. The capsule of the cyst is in direct continuity with the muscular and adventitial wall of the bladder below. The cells of the solid portion invade the musculature of the bladder directly.

The consistency of the mass is usually fluctuant, since it is mainly composed of multiloculated spaces separated by thin septums and containing a clear gelatinous viscid fluid. The upper part of the tumor lies

13. Kielleuthner, L.: Ztschr. f. Urol. **23**:519, 1929.

14. Pendl, F.: Beitr. z. klin. Chir. **19**:681, 1914.

between the transversalis fascia and the peritoneum of the anterior abdominal wall near the midline. Usually it is adherent to the peritoneum, and occasionally it breaks through this layer, forming adhesions with the abdominal contents. Frequently the tumor may not be in the midline but on the right or the left because of pull by the lateral umbilical ligaments. The upper end may be pulled laterally and downward so that it takes a course from the apex of the bladder upward and outward to the right or to the left.

The capsule of the upper portion of the tumor is fairly definite. Of variable thickness, it may be composed of fibromuscular tissue or old hyalinized connective tissue showing calcareous deposits. There are usually vestiges of acini on the inner surface, evidence of the fact that the capsule is merely compressed tumor.

The stroma consists of fibrous bands of varying thickness which converge in the center. It may show hyalinization and calcification. It encloses spaces varying in magnitude from microscopic ones to huge cysts.

Microscopically, the surface of the tumor, the cysts and the acini are lined by cylindric cells showing various phases of mucin production or mucinous degeneration. They are frequently pleomorphic and frankly carcinomatous and reproduce by amitotic division. While the general appearance of the tumor bears a strong resemblance to rectal cancer, definite differences exist which permit differentiation between rectal and apical cancer. Rectal cancer as a rule produces a more developed and permanent type of granular cell which holds its form longer and is more stable than that of a tumor of the urachus. In the latter, so great is the production of mucus and so rapid the breakdown of the cells that the formed elements occur over a very limited area. The cell goes through its cycle of mucin production and is soon destroyed by its own activity. It is crushed out of existence before it can take part in the fantastic riot and vicious activity of its fellows.

#### CLINICAL HISTORY

Usually complaints due to the growth of the tumor are elicited before any disturbance in micturition ensues. Owing to interference with the action of the urachus, there is some hesitancy in starting the stream, and there is mild frequency. The first complaint is usually abdominal discomfort and tenderness on palpation in the suprapubic region, where a mass may be seen or felt. Often the first symptom is hematuria with frequency which, owing to inflammation, is usually associated with pain on micturition. Later, pieces of tumor or a glairy mucoid substance may be found in the urine. Inflammatory phenomena may mask the hematuria, since, as in all cases of cancer of the bladder, infection is likely to set in early.

## DIFFERENTIAL DIAGNOSIS

Because of the suprapubic mass, one should consider neoplasms of the omphalomesenteric duct, echinococcic cysts, abscess in the space of Retzius, lipoma and cyst arising within the abdominal cavity.

## CYSTOSCOPIC APPEARANCE

Tumor of the urachus may be present merely as a protrusion or swelling of the apex of the bladder, giving the impression that something is pressing from the outside. There may be merely what appears to be a flat epithelioma. In other cases there may be a funnel-shaped retraction of the apex of the bladder with a ring of tumor around it. In others, there may be papillary or polypoid masses projecting into the bladder. Occasionally gelatinous fluid may be passing into the bladder. Induration is usually present.

## AGE INCIDENCE

In Beggs's series no tumor occurring in a patient younger than 29 was observed, and the majority appeared after middle life. In the same series, 14 tumors occurred in males and only 4 in females.

## TREATMENT

Metastases are rare, but they are relatively frequent after surgical intervention. From this it is apparent that any operation must be radical. Metastases following operation occur in the space of Retzius; therefore, the operative specimen should include the umbilicus, a wedge-shaped block of the transversalis fascia, the peritoneum and at least the upper half of the bladder.

## PROGNOSIS

There is no evidence concerning the cancerous potentiality of the untreated tumor. The prolonged history in cases of cancer indicates that cancer has superimposed itself on a noncancerous tumor rather than that the tumor is of relatively low malignancy. None of the patients observed lived more than two and a half years after operation. Immediate operative results are good.

## REPORT OF A CASE

A white man, aged 26, in 1940 complained of pain in the lower right quadrant of the abdomen suggesting appendicitis. In March 1943 he began to pass bloody urine which contained a glairy mucoid exudate. He experienced intermittent episodes of painless hematuria for four to five months, after which stranguria, urgency and daily hematuria began. In spite of this, it was not until May 1944 that he was admitted to a station hospital. On cystoscopy, a tumor about the size of a small apple, which had bits of necrotic tissue and calcareous plaques attached to it, was seen on the posterodextrolateral wall of the bladder, allegedly 10 cm. above the

right ureteral orifice. A cystogram showed an irregular filling defect and calcification in the right upper third of the bladder wall. The diagnosis was probable papillary carcinoma of the bladder.

The patient was transferred to a general military hospital in June 1944, over four years after the onset of symptoms. A grapefruit-sized mass was palpable in the suprapubic region. This extended to within two and a half fingerbreadths of the umbilicus and was situated somewhat to the right of the midline. The mass was exquisitely tender to palpation, and there was rebound tenderness. On rectal examination a doughy mass the size of a tennis ball was palpable above and to the right of the prostate. Urinalysis showed large numbers of red blood cells and pus cells. Blood counts revealed moderate anemia and some increase in leukocytes.

Intravenous pyelography and cystography showed a flat depression of the superior portion of the bladder with a slight convexity, which was attributed to pressure from a mass external to the bladder. There was some dilatation of the upper portion of the right ureter and the renal pelvis, indicative of obstruction. The belief was entertained that the patient had an old appendical abscess involving the lower right quadrant of the abdomen and the pelvis, which caused the bladder defects.

Cystoscopy substantiated the diagnosis of tumor of the bladder, however, since a sessile cauliflower-like mass, measuring 3.5 to 4.0 cm. in diameter and projecting 1.5 cm. into the lumen, could be visualized in the dome of the bladder near the insertion of the urachus. A specimen of urine collected at that time contained a large amount of mucoid material in which large numbers of pleomorphic cells with irregular-shaped nuclei were present. These were diagnosed as cancer cells, type undetermined.

On operation a grapefruit-sized tense, fluctuant, cystic tumor was found firmly attached to the anterior abdominal wall in the space of Retzius and between the parietal peritoneum and the transversalis fascia. The mass was firmly adherent to the dome of the bladder, where its inferior pole formed an apple-sized firm mass in the bladder wall. A circumferential incision was made in the normal portion of the vault of the bladder wall outside of the intravesical tumor. The cyst was stripped away from the abdominal wall, but unfortunately it was incised, and a large amount of hemorrhagic mucinous material escaped. The posterior portion of the cyst wall could not be removed and was left in situ.

The cystotomy wound healed slowly but gradually closed over. The patient was able to void spontaneously. He became afebrile, gained weight and was quite comfortable. Sigmoidoscopic examination was performed about one month after the operation, and no neoplastic alterations of the mucosa of the rectum or the sigmoid were noted. There was no bloody discharge, nor could any relationship between the fundus of the bladder and the sigmoid or the rectum be demonstrated. Four months after admission the patient was transferred to another general hospital for high voltage roentgen therapy.

At that time the suprapubic wound was almost completely healed. There was a firm, smooth-surfaced, slightly tender mass approximately the size of a lemon in the left lower quadrant of the abdomen above and parallel to the inguinal ligament. The impression was that there were palpable nodules along the anterior margin of the liver. There was no generalized lymphadenopathy. Cystoscopy revealed no deformity or infiltration anywhere. Anteriorly the suprapubic scar could be seen. It was somewhat indurated, and there was some suggestion of recurrence, because of the presence of a small nodule measuring 3 mm. in diameter.

Revisualization several weeks later proved that a yellow lesion was present, which was too small for biopsy, situated in the distal portion of the old suprapubic scar.

Intravenous cystography showed some flattening of the left superior quadrant of the bladder associated with some lateral displacement of the left ureter near the margin of the bladder. Retrograde pyelography showed that both ureters were quite large and also showed the lateral displacement of the left ureter. The belief was expressed that a mass might be present which was causing this displacement. No abnormalities of the contour of the bladder were evidenced on opaque and air cystography.

Repeated roentgenologic examination of the skeleton and the lungs failed to demonstrate any metastatic involvement of these organs.

The patient's condition at the time of writing (June 1945) is quite satisfactory. He is ambulatory, weighs 160 pounds (72.5 Kg.) and has no genitourinary complaints. Because of the presence of the mass in the left lower quadrant of the abdomen and the evidence of displacement of the left ureter, local recurrence or metastases is suspected.

*Pathologic Report.*—(a) Gross Appearance of Specimen: The operative specimen consists of the vault of the bladder comprising a grapefruit-sized supravesical cystic structure and an apple-sized solid intramural portion. The intramucosal aspect (fig. 1) measures approximately 4.5 cm. in diameter. The central portion consists of a nodular, raspberry-like, red soft polypoid tumor which projects above the surface. Many small crypt openings, from which well large amounts of mucoid substance, may be seen between the nodules. Between the crypts the surface of the nodule is lined by discolored, ulcerated mucosa. Other portions of the tumor possess a villous, friable, gelatinous appearance. A smooth, pale, occasionally indurated seam of bladder mucosa measuring 0.5 cm. in width surrounds this central tumor. The wall of the supravesical cyst merges with the adventitia and the muscle coat of the bladder. The cyst wall measures approximately 3 to 4 mm. in thickness. Its external surface is smooth and glistening, while its internal aspect is somewhat rough and nodular and appears to be lined by accumulations of nonadherent, crumbling gelatinous material. The cyst is not entire, a wedge-shaped segment of wall from the posterior aspect being absent.

The intracystic part of the tumor is composed of a papillary, lobular, friable, hemorrhagic, necrotic, gelatinous mass which forms a raised branching stalk that projects into the lumen of the cyst. In the center of this stalk one notes a white-gray, rather firm fibrous plaque, in the center of which is a small circular opening. Serial sagittal sections of the tumor (fig. 2) disclose almost complete honeycombing throughout. Large multiloculated spaces are distended with clear, white, gelatinous material separated by irregularly shaped and sized fibrous septums. On one side the muscle coat is completely replaced by the colloid tumor, whereas on the other, the smooth muscle of the bladder is markedly thickened, firm and hyperplastic, although infiltrated to some extent by tumor. Coursing perpendicularly, although inclined somewhat toward the right, is the urachus, the thick fibrous walls of which are quite sharply defined; from its outer surface radiate septums forming the connective tissue stroma of the mass. The lumen contains an elongated calcified plug, which is rather firmly attached to the left side of the wall. Between the wall and the plug there is a definite space filled with mucinous debris. On serial sections this tubule can be seen to communicate between the urinary bladder and the lumen of the supravesical cyst, the opening of which was noted earlier in this paragraph. Diffuse areas of hemorrhage are seen throughout.

(b) Microscopic Appearance: Sections of the intramucosal portion of the tumor reveal the usual transitional epithelium of the bladder, which invaginates rather sharply into a crypt where it forms tubular and branching tubular mucous glands (figs. 4 and 5). These are lined by a rather regular row of columnar epithelium, in

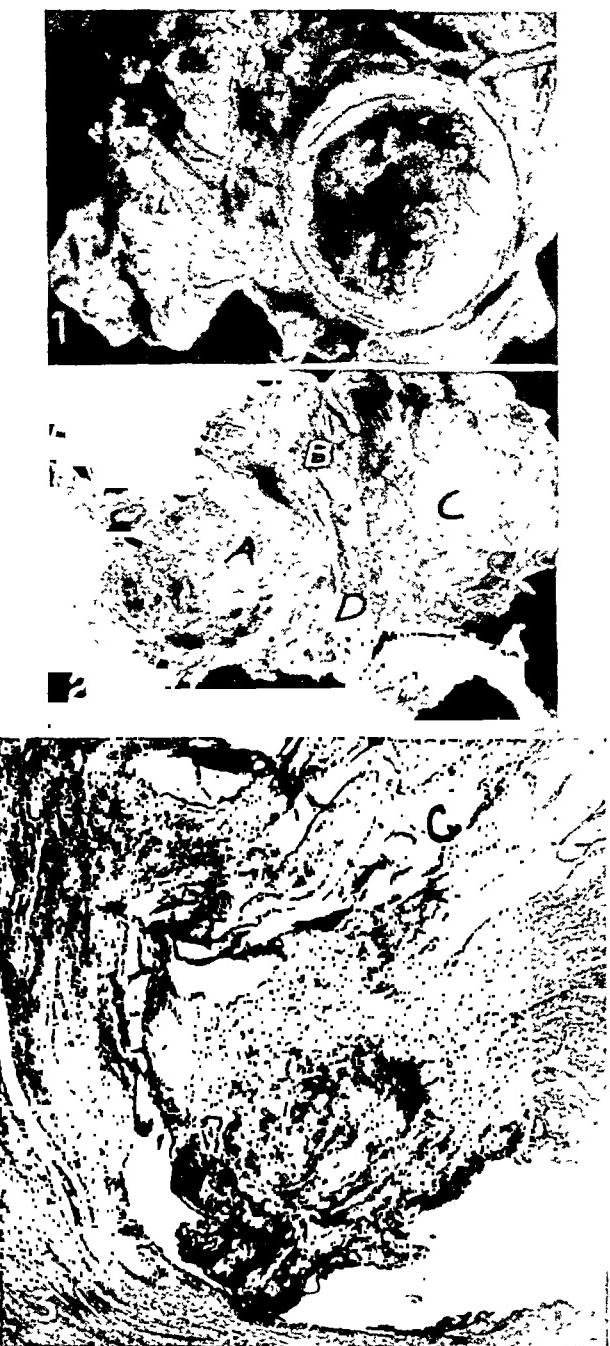


Fig. 1.—Photograph of the gross specimen showing the intravesical aspect of the neoplasm with the central nodular tumor surrounded by a seam of normal bladder mucosa.

Fig. 2.—This photograph represents a sagittal section made through the center of the tumor. *A* demonstrates the thickened, hypertrophied musculature of the bladder; *C*, the multiloculated, colloid-filled, cystic degenerated muscle tissue; *B-D*, the portion of the exposed urachal canal containing a calcified plug; *F*, a reflection of the supravesical cyst wall.

Fig. 3.—Photomicrograph of a cross section of the urachus, taken midway between *B* and *D* in figure 2. This shows the well developed wall on one side and a portion of the lumen, in which a dense fibrous plug, showing considerable calcification, is present. Extending laterally are the thin, irregularly shaped septums enclosing colloid-filled spaces. Rudimentary, flat epithelium can be observed in several places. ( $\times 26$ .)

which many goblet cells may be seen. Most of the cells possess large oval nuclei, basally located. The greatest portion of the cell is usually transformed into dense mucinous material, which is poured into the crypt. Frequently the transitional epithelium reappears between the crypt openings on the surface of the tumor and disappears in another invagination. The deeper portions of the mass consist of large, multiloculated, cystic structures, frequently lined by a single layer of columnar cells as well as short cuboidal cells; all the cysts display advanced colloid degenera-

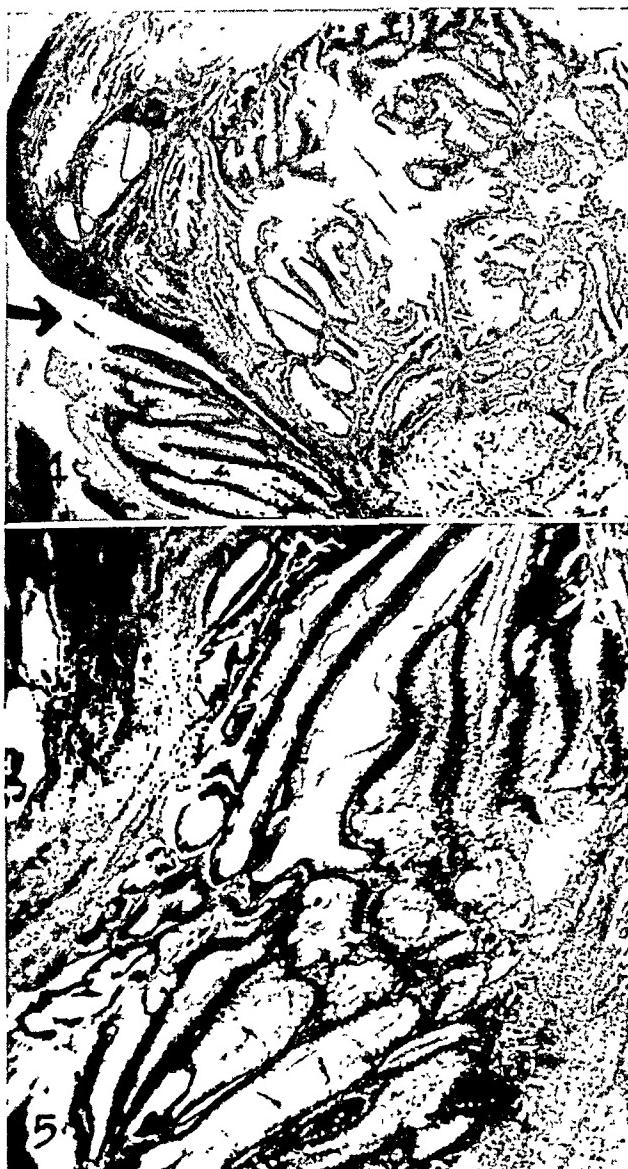


Fig. 4.—Photomicrograph of the surface of the intramucosal portion of the tumor disclosing the papillary, filigree-like arrangement of the epithelium, forming colloid-filled acini. The arrow indicates a crypt opening lined by transitional epithelium, below which are well formed mucous glands. ( $\times 16$ .)

Fig. 5.—Photomicrograph showing highly differentiated glandular acini lined by tall columnar epithelium. The acini contain considerable mucinous material, and evidence of secondary infection may be observed. ( $\times 112$ .)

tion, and the cavities are plugged with mucin. The septums are formed by thin, slender connective tissue bands which branch irregularly throughout the muscle and the submucosal coats. Many stages in the development of colloid carcinoma can be seen between well developed acini and pools of mucin possessing no cellular lining. The latter characteristic appears to predominate as one approaches the supravesical portion of the tumor, where the growth is far more irregular and disorderly. Longitudinal and cross (fig. 3) sections of the urachus disclose the presence of a definite lumen containing dense, calcified fibrous tissue showing some evidence of bone formation. At times the tubule possesses a rudimentary cuboidal epithelium, and at other times the epithelium is characteristically mucinous and columnar. There is marked hypertrophy of the smooth muscle layer, and a rather sharp line of demarcation is observed between it and the bulk of tumor on one side. On the other, however, the muscle layer is completely disintegrated by the irregular infiltration of branching glandular acini. There is severe granulocytic infiltration of the duct and its surrounding structures. Occasionally one finds accumulations of these cells leading to the formation of microscopic abscesses. The wall of the supravesical cyst is devoid of epithelium or of mucin-producing elements, although mucin is occasionally seen attached to its internal aspect.

#### COMMENT

The history of long-standing vague abdominal complaints, followed by hematuria and increasing signs of pathologic change in the lower genitourinary tract, associated with the cystoscopic appearance of the lesion, is quite characteristic of this tumor. The pathologic appearance of adenocarcinoma of the urachus and the lack of relationship of the tumor and the rectum or the colon are considered proof of the genesis of this neoplasm. It is interesting to note that the patient is the youngest on record. The case conforms otherwise, in all respects, to the criteria laid down by previous authors.

#### SUMMARY

Primary adenocarcinoma of the urachus involving the vault of the bladder occurred in a 26 year old man.

# TERATOMA OF THE ANTERIOR MEDIASTINUM IN THE GROUP OF MILITARY AGE

A Study of Sixteen Cases, and a Review of Theories of Genesis

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THE study of teratoma in the light of modern embryology has been neglected by American pathologists. The purpose of this paper is to present an interpretation of the origin and the development of teratoma, with particular reference to that of the anterior mediastinum. The material for the study is provided by a series of 16 specimens of teratoma from 15 men and 1 woman within the military age group of 18 to 38 years.

The outstanding achievements of the experimental embryologists pertinent to our subject can be summarized briefly. In 1891 Driesch<sup>1</sup> observed that each of the first two or four blastomeres of a sea urchin's egg, if separated by shaking, can develop into a whole embryo. Three years later O. Schultze<sup>2</sup> found that when a fertilized frog ovum in the two cell stage was inverted a whole embryo developed from each of the two blastomeres. In 1929 Penners demonstrated that even after the four or the eight cell stage had been reached the experiment of Schultze would yield as many embryos as there were cells. The finding of such totipotency in amphibian blastomeres led to investigations on the fate of transplants exchanged between blastulas or early gastrulas. In this field the work of Spemann<sup>3</sup> and his students is prominent. It soon became apparent that before gastrulation the fate of each of the various regions of an embryo has not been determined. Thus, if a lateral piece of the blastula (presumptive skin) is excised and transplanted into the region of the presumptive neural plate of another blastula, it will form neural plate, not skin. Likewise, presumptive neural plate will form skin if transferred to the lateral region of the blastula. Similar results may even be obtained when tissue exchanges are made between regions that later develop into different germ layers. Thus, presumptive skin transplanted to the dorsal lip of the blastopore (presumptive mesoderm) is invaginated along with the surrounding cells to form mesodermal somites

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From the Army Institute of Pathology, Washington 25, D. C.

1. Driesch, H.: *Ztschr. f. wissenschaftl. Zool.* **53**:160, 1891.

2. Schultze, O.: *Arch. f. Entwicklungsmechn. d. Organ* **1**:269, 1894.

3. Spemann, H.: *Embryonic Development and Induction*, New Haven, Conn., Yale University Press, 1938.

and notochord. This exchange of cell groups between different presumptive germ layers has been widely interpreted as evidence for the non-specificity of the germ layers.<sup>4</sup> However, a timely note of caution has been expressed by McCrady,<sup>5</sup> who emphasized the need for making a clear distinction between germ layers and presumptive germ layers: the latter are precursors of the former, but they are not identical with them. Hence, interchangeability of the presumptive germ layers does not signify that the germ layers themselves can be interchanged.

After completion of gastrulation the results of exchange transplantsations are quite different. At these later stages the fate of each of the various regions has been determined, and the transplant follows its course of development uninfluenced by the new surroundings. A presumptive eye region if placed in the belly wall becomes an eye, not skin or muscle. The first region whose fate is determined is the presumptive mesoderm in the dorsal lip of the blastopore. If transplanted to an area of presumptive epidermis it does not form skin but sinks beneath the surface and there develops into somites or a notochord. After the implant has sunk beneath the surface a surprising phenomenon occurs; the overlying ectoderm, which normally would have produced skin, gives rise to a neural plate or even a secondary embryo. In other words, the implant has "induced" the formation of a neural plate. That is precisely what happens during normal development. Gastrulation consists in a large measure of a rolling in of presumptive surface mesoderm at the blastopore lip. The mesoderm then comes to lie immediately beneath the presumptive ectoderm, in which it "induces" the formation of a neural plate. The presumptive mesoderm has therefore been called the "primary organizer" of the amphibian embryo.

During the decade preceding the outbreak of the second world war, considerable progress was made in investigating the nature of the intra-cellular substance which acts as the organizer. In 1931 Spemann showed that crushing the cells had no deleterious effect on their inductive activity. In the following year several investigators working independently found that boiling the cells likewise did not affect their ability to induce organization. In fact, Holtfreter<sup>6</sup> demonstrated that after boiling, parts of the gastrula which formerly possessed no ability for induction now had acquired it. In 1933 the same investigator announced the discovery that adult tissues of all phyla will induce the formation of secondary embryos if implanted into amphibian blastulas.<sup>7</sup> Cell-free filtrates and numerous synthetic organic compounds have likewise induced the formation of

4. (a) Oppenheimer, J. M.: Quart. Rev. Biol. **15**:1, 1940. (b) Needham, J.: Biochemistry and Morphogenesis, London, Cambridge University Press, 1942.

5. McCrady, C., Jr.: J. Tennessee Acad. Sc. **19**:240, 1944.

6. Holtfreter, J.: Arch. f. Entwicklungsmechn. d. Organ **132**:225, 1934.

7. Holtfreter, J.: Naturwissenschaften **21**:766, 1933.

complex structures. Needham<sup>4b</sup> concluded that the natural organizer is a steroid, since besides the evidence of its chemical and physical characteristics "the only substance which so far has been shown to act in concentrations of the vitamin or hormone order is a polycyclic hydrocarbon."

The term "primary organizer" implies the existence of secondary and tertiary organizers. In several instances these had been identified before the concept of the primary organizer developed. The action of successive and increasingly specific organizers has been most completely elucidated for the eye. The primary organizer induces the formation of a neural plate which in turn becomes the central nervous system. Optic vesicles develop as lateral evaginations of the forebrain; subsequently they invaginate and are known as optic cups. The latter act as secondary organizers in that they induce the formation of a lens in the overlying ectoderm. If the optic cup is removed, no lens is formed; if displaced so that it lies beneath a portion of the ectoderm which normally becomes skin, the ectoderm will form a lens. A tertiary organizer is present in the lens and is responsible for the induction of changes in the overlying ectoderm leading to the formation of the transparent cornea.

In this brief summary the fascinating byways and perplexing problems of the subject cannot be considered. An excellent review of the chemical and physical methods of investigating embryogenesis, as well as a critical historical survey of developmental morphologic research, has been written by Needham.<sup>4b</sup> The implications of these studies for the understanding of teratoma will be considered in a subsequent section.

#### MATERIAL

The material on which this report is based comprises the clinical records and specimens of 16 cases of teratoma of the anterior mediastinum reviewed during the past four years at the Army Institute of Pathology.<sup>8</sup> Fourteen of the patients were soldiers, 1 was the wife of a soldier and 1 was a male civilian. Since all but the last were encountered in army hospitals, the preponderance of the male sex is of no significance. In view of the several million men under arms, every one of whom has had at least one routine roentgenologic examination of the chest, the incidence of mediastinal teratoma is low. Rusby<sup>9</sup> was able to find reports of 245 cases of teratoma in the literature at the end of 1939 and added a report of 6 of his own. In the present study the cases are arranged in two groups: In the first group, 10 cases, the tumor was benign; in the remaining 6 it was cancerous.

#### GROUP I. BENIGN TERATOMA

Of the 10 patients of this group, the oldest was 36 years of age, the youngest 20; the average age was 24 years. Two patients (cases 3 and 8) were wholly without signs or symptoms, the growths being discovered

8. An additional case has been previously reported by S. J. Wilson and R. Cares (Arch. Path. 39:113, 1945).

9. Rusby, N. L.: J. Thoracic Surg. 13:169, 1944.

in routine roentgenograms of their chests. The other patients had symptoms for periods ranging from two weeks to two years; the average duration was approximately four months. In 5 patients the presenting symptom was pain in the chest, followed by cough and dyspnea; 3 patients complained of shortness of breath and cough before they experienced any pain. Seven patients recovered completely after operative removal of their tumors. Two died soon after operation; a third died from compression of mediastinal structures.

#### REPORT OF CASES

**CASE 1.**—*Clinical Course.*—A 23 year old white soldier complained of a deep pressing ache in the right side of his chest about September 1942. Roentgenograms of the chest taken at that time were interpreted as showing cardiac enlargement, a diagnosis that was repeated when similar studies were made the following year. The thoracic pain recurred at intervals, was located to the right of the sternum between the third and the fourth rib and did not radiate. In August 1944 an acute

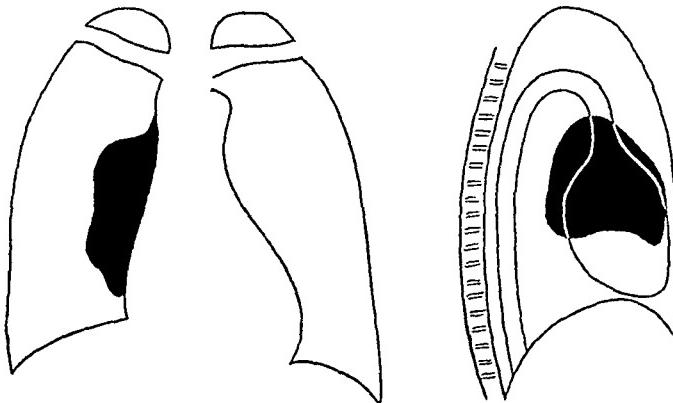


Fig. 1 (case 1).—Diagram of the roentgen shadows in the right lung field.

The outline drawings which illustrate the cases are copied from representative roentgenograms of the chests of the patients. The part of the tumor that encroached on the lung field is shown in solid black, the portion in the precordial region cannot be distinguished from the shadow of the heart and large vessels. If the tumor outline is indefinite, this will be indicated by cross hatchings; fluid in the pleural cavity is represented by horizontal lines. In a few figures, both frontal and lateral views are given.

febrile illness developed, and at that time roentgenograms of the chest revealed a circumscribed dense homogeneous shadow, 5 by 7.5 by 10 cm., at the anterior medial border of the right lung field (fig. 1.). Cutaneous tests with coccidioidin, tuberculin and hydatid cyst fluid were negative. At operation, two months later, a large cystic tumor was found in the anterior mediastinum, attached to the pericardium and displacing the hilus of the right lung. The firm pericardial attachment of the tumor made sharp dissection impossible, necessitating piecemeal removal. Post-operative recovery was uneventful.

*Gross Examination.*—The specimen consisted of several large pieces of cyst wall, 2 to 5 mm. in thickness. The lining surface was granular and red, owing to scattered deposits of old blood pigment.

*Microscopic Examination.*—The cyst wall consisted of dense, partly hyalinized connective tissue. Attached to the outer surface were small masses of thymus (fig. 2C). The following components were recognized:

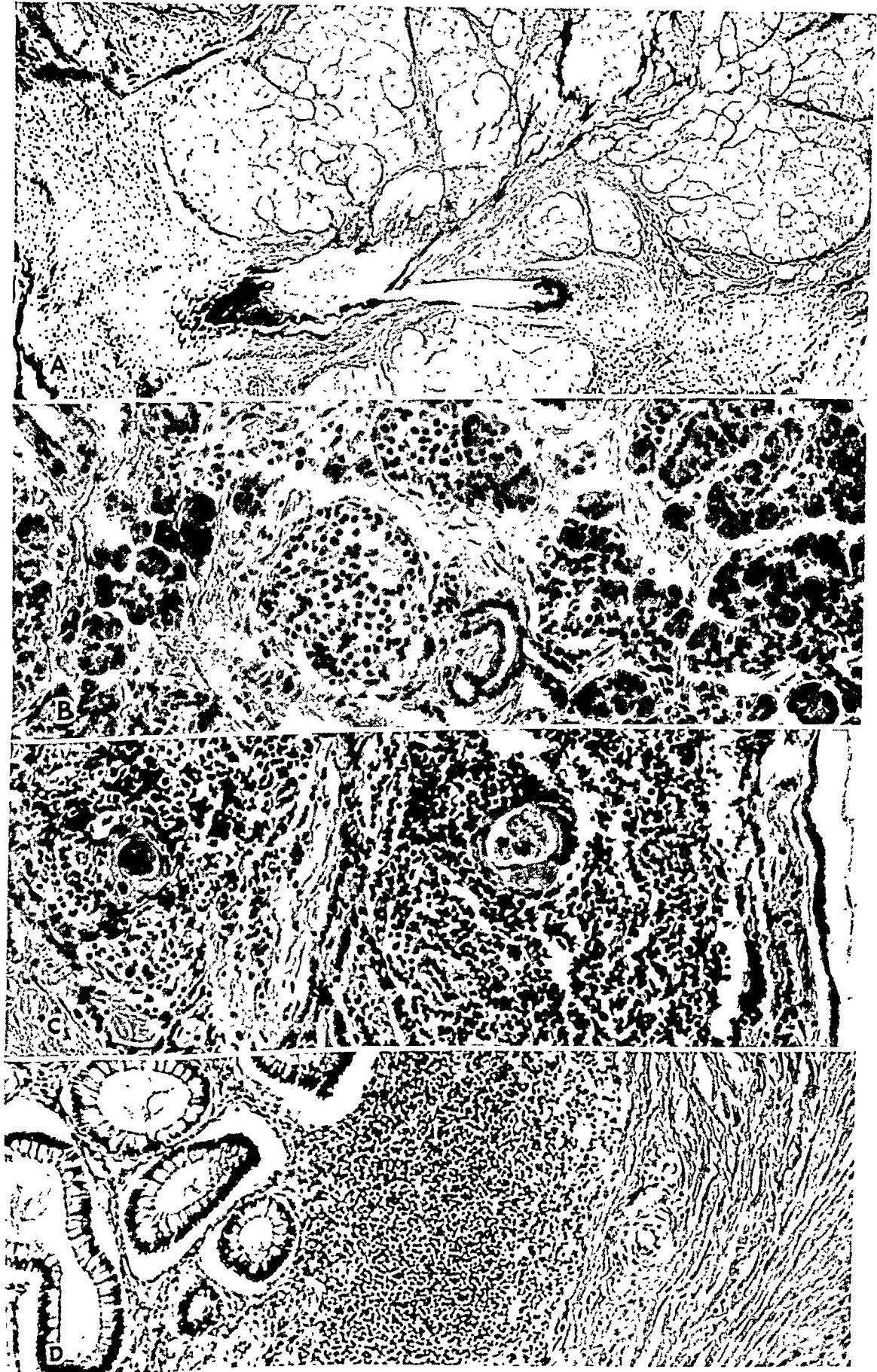


Figure 2  
*(See legend on opposite page)*

(a) Ectodermal Derivatives: Most of the lining epithelium of the cyst had disappeared; however, in some areas stratified squamous epithelium remained. Associated with this were hair follicles, sebaceous glands (fig. 2A) and sweat glands.

(b) Mesodermal Derivatives: Several isolated masses of smooth muscle were present. Scattered islands of fat cells separated the bundles of connective tissue. The blood vessel walls were well developed. Hyaline cartilage was found associated with ciliated epithelium.

(c) Entodermal Derivatives: Ciliated columnar epithelium formed the lining of structures resembling bronchi. These contained islands of cartilage and mucous glands; the acini of the latter often contained demilunes of serous cells. Pancreas with well developed acinous tissue, dilated ducts and islets of Langerhans were abundant (fig. 2B).

*CASE 2.—Clinical Course.*—A 23 year old white soldier was admitted to the hospital for treatment of a shrapnel wound of the right leg. During convalescence he complained of pain in the chest. A roentgenogram revealed a large mass in the upper part of the anterior mediastinum with some evidence of tracheal compression. Surgical removal of the tumor was undertaken; although it was adherent to the pleura and the aortic arch, the mass was freed and removed intact. While the wound was being closed there was sudden profuse bleeding from the aorta. Attempts to control the hemorrhage failed, and the patient died.

*Gross Examination.*—The tumor measured 8 by 5 by 5 cm., had a smooth, somewhat lobulated surface, and on section was found to contain many cystic spaces filled with a clear viscid fluid. The cysts were separated by firm fibrous tissue containing several areas that were cartilaginous in consistency.

*Microscopic Examination.*—Only three blocks of tissue were available for histologic examination; the rest of the material was lost in transit as a result of enemy action.

(a) Ectodermal Derivatives: No tissue of this germ layer could be recognized with certainty. Small patches of squamous cell epithelium were probably metaplastic derivatives of the columnar ciliated epithelium with which they were associated.

#### EXPLANATION OF FIGURE 2

A, section of the lining epithelium of the large cyst (case 1), showing differentiation into skin and its appendages. At the lower left is a portion of stratified squamous epithelium of the surface which extends inward about the shaft of a hair. The hair is cut tangentially; only the basal portion of the shaft and the well developed hair follicle are shown in the section. A large sebaceous gland opens into the connective tissue sheath of the hair. Masson's trichrome stain;  $\times 100$ .

B, pancreatic tissue (case 1) containing both islets of Langerhans and acinous cells. At the center are two islets. Just below and to the right of center is a prominent duct. Darkly staining acini and supporting stroma occupy the remainder of the figure.  $\times 500$ .

In C, on the extreme right, is shown the surface of the cystic teratoma (case 1). Incorporated in the wall is thymic tissue, recognizable in the figure as collections of lymphocytes containing two Hassall's corpuscles.  $\times 230$ .

D, structure resembling large intestine (case 3). The "mucosa" is made up of tubular glands lined by goblet cells. A distinct "muscularis mucosae" is not present. In the narrow "submucosa" is a collection of lymphoid tissue, beneath which lie circular bundles of smooth muscle.  $\times 145$ .

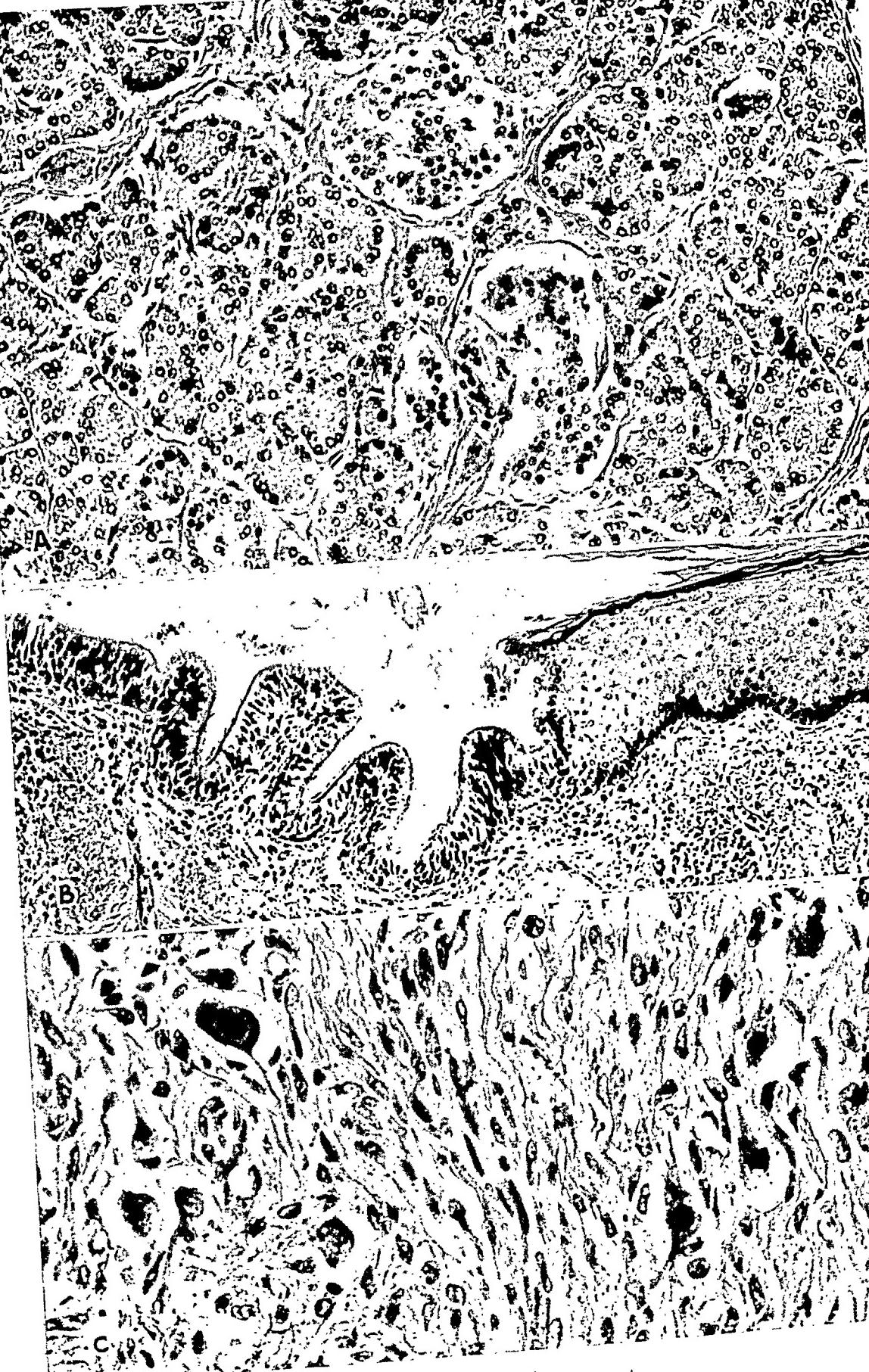


Figure 5  
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**CASE 4.—Clinical Course.**—A 23 year old white soldier began to have symptoms of illness nine months before his death. These consisted of attacks of severe epigastric pain and vomiting, occasionally associated with a sensation of squeezing about his heart. He began to lose weight and felt chronically ill. A roentgenogram of the chest made a month after the onset of symptoms revealed a tumor in the anterior mediastinum (fig. 6). High voltage roentgen therapy with a total dosage of 1,000 roentgens (r) produced no change in the lesion. Thoracoscopy, carried out at the fifth left interspace, disclosed a smooth, round, yellowish tumor, apparently arising in the anterior mediastinum. An exploratory thoracotomy with approach through the left side of the chest confirmed these findings and likewise demonstrated firm union of the pericardium with the tumor. Removal of the mass was not attempted at that time. After the wound had healed, thoracotomy was performed by way of the right side of the chest. The tumor was tense and cystic; 700 cc. of thin milky fluid was aspirated before removal was attempted. The lower portion of the neoplasm was readily separated from the surrounding tissue, but in the region of the thymus the adhesions were so dense that not all of the tumor could be excised. During the operation the patient went into shock, from which he only partially recovered. After a few hours his temperature rose, convulsive movements of his left arm occurred, and he died.

**Gross Examination.**—The specimen consisted of an irregular, ragged cyst wall, from which fragments of tissue had been cut or torn during removal. It measured approximately 13 by 6 cm. and was 2 mm. in thickness. Near one pole the wall was thickened as a result of the presence of many firm nodules, some of which were covered by hair-bearing "skin."

**Microscopic Examination.**—(a) Ectodermal Derivatives: Many cysts were lined by squamous epithelium, often associated with hair shafts, sebaceous glands and sweat glands. In several regions the "skin" was abruptly replaced by ciliated columnar epithelium (fig. 5B).

(b) Mesodermal Derivatives: Bundles of smooth muscle were found in association with the "skin" or the ciliated epithelium, beneath which were occasional collections of lymphoid tissue containing well differentiated germinal centers. Adipose tissue appeared in irregular islands that had no definite relation to surrounding structures.

(c) Entodermal Derivatives: Ciliated columnar epithelium lined several small cysts. Occasionally, organoid respiratory structures were found to contain mucous glands. Prominent in several sections were large masses of pancreatic acinous tissue associated with dilated ducts and islets of Langerhans.

**CASE 5.—Clinical Course.**—About November 1942 a 29 year old white soldier first noticed throbbing pain in the precordial region after exercise. These attacks became more frequent and of longer duration, accentuated by an accident in which the patient wrenched his back. A roentgenogram of the chest taken at this time (September 1943) revealed an intrathoracic tumor. Roentgen therapy to a total

#### EXPLANATION OF FIGURE 5

A, well differentiated pancreas (case 3). At the center are two islets of Langerhans.  $\times 230$ .

B, wall of a small cyst lined by pseudostratified ciliated columnar epithelium and stratified squamous epithelium (case 4). The transition between the two epithelia is abrupt.  $\times 160$ .

C, neuroglia and ganglion cells, some of the latter with visible axis-cylinders and dendrites. (case 6). Bodian's silver-aniline blue stain;  $\times 500$ .

dosage of 1,000 r was given without apparent effect on the size of the lesion. A diagnosis of teratoma of the anterior mediastinum was made. In March 1944, a year and four months after the onset of symptoms, a thoracotomy was performed and the tumor excised. In August 1944 the patient was transferred to a convalescent center with recommendation for return to duty.

*Gross Examination.*—The tumor, which measured 7.5 by 6.5 by 6 cm., was well encapsulated and fluctuant. The wall, 5 mm. thick, enclosed a space filled with sebaceous material and hair. The smooth lining surface bore several nodules from which arose long hairs.

*Microscopic Examination.*—(a) Ectodermal Derivatives: These were represented by skin with its appendages of hair, sebaceous glands, sweat and apocrine glands.

(b) Mesodermal Derivatives: These were represented by adipose and connective tissue from the supporting stroma of the cyst wall. Beneath the epidermis were many parallel bundles of smooth muscle. Large lymphatic channels were prominent in several sections. Collections of lymphoid tissue were likewise present. Areas of necrosis in which no structural details were recognizable were attributable to pre-operative irradiation.

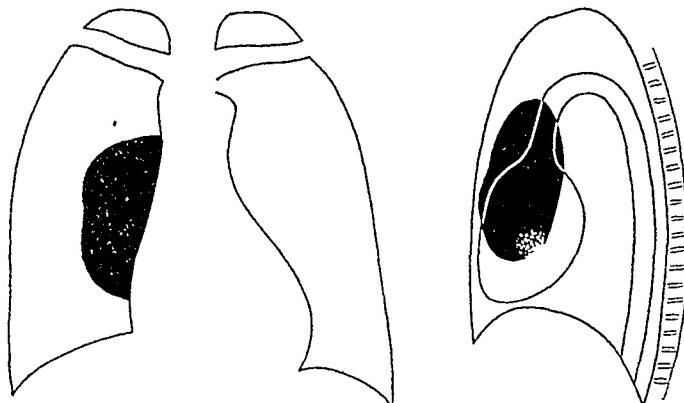


Fig. 6 (case 4).—Diagram of the roentgen shadows in the anterior mediastinal field.

(c) Entodermal Derivatives: Several cysts were lined by columnar ciliated epithelium, beneath which bundles of smooth muscle were frequently found. However, more complex organoid respiratory structures containing cartilage and mucous glands were absent.

**CASE 6.—Clinical Course.**—A 22 year old white soldier was well until August 1941, when fleeting pains began to occur in the upper anterior part of the left side of the chest and the left arm. Four months after the onset of these symptoms, which had become progressively more severe, the patient became dyspneic and began to have a moderate, nonproductive cough. A roentgenogram of the chest revealed a well circumscribed tumor arising in the anterior mediastinum and encroaching on the hilus of the left lung (fig. 7). At operation (February 1942) a cystic mass was found densely adherent to the pericardium; it extended laterally as far as the nipple line and displaced the left lung posteriorly. To the right it passed beyond the median line of the sternum and extended down to the fifth intercostal space. The superior border of the lesion passed along the arch of the aorta. Recovery was uneventful.

*Gross Examination.*—The tumor was spherical and cystic and measured 10 by 10 by 12 cm. The surface attached to the pericardium was roughened; elsewhere

it was smooth and glistening. It varied in color from yellowish pink to white. The smooth surface was interrupted by six scattered nodules varying in diameter from 1 to 4 cm., with a maximum elevation of 1.5 cm. On the side opposite the pericardial attachment there was a cordlike structure 9 cm. in length and 0.5 cm. on cross section. Histologic examination showed this to be normal thymus gland. The cut surfaces of the tumor were composed of lobules of fatty tissue separated by strands of white fibrous tissue that blended into cartilage. Cysts, ranging from 1 to 15 mm. in diameter, with rather thick walls, were scattered through the mass. Some of the larger cysts contained sebaceous material, which in one was mixed with hair.

*Microscopic Examination.*—(a) Ectodermal Derivatives: Many of the cysts were lined by skin bearing well differentiated hair follicles, sebaceous glands and sweat glands. In one region there were numbers of ganglion cells with well developed axons and dendrites surrounded by neuroglia (fig. 5C).

(b) Mesodermal Derivatives: Hyaline cartilage, usually in close association with ciliated columnar epithelium, was abundant. In some regions the cartilage cells had taken axial positions and were associated with endochondral bone formation (fig. 8A). Between the interstices of this bone there was active hemopoietic

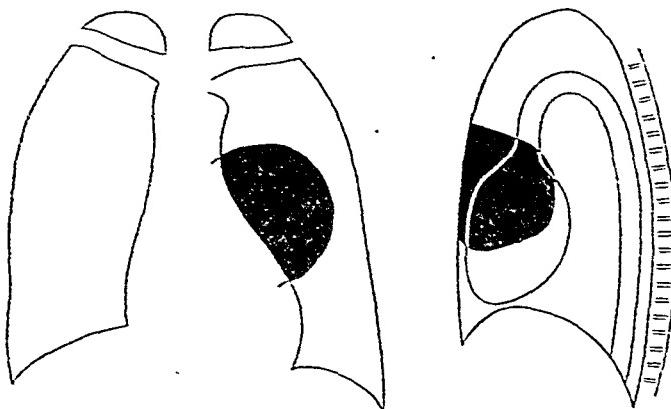


Fig. 7 (case 6).—Diagram of the roentgen shadows in the anterior mediastinal field.

tissue. Membranous bone formation was also present in several areas (fig. 8B and C). Lymphoid tissue with occasional follicle formation was frequently seen adjacent to "intestinal" and "bronchial" epithelia. The latter were often surrounded by smooth muscle; in other regions smooth muscle had formed large masses unassociated with epithelial structures (fig. 8D). Adipose tissue was scattered throughout the sections.

(c) Entodermal Derivatives: Many spaces were lined by ciliated columnar epithelium, which in turn was surrounded by hyaline cartilage, smooth muscle and mucous and serous glands—a fair reproduction of a bronchial wall. Similarly, mucous epithelium, associated with smooth muscle and lymphoid tissue, duplicated a section of the large intestine (fig. 9A). Masses of dark-staining epithelial cells, containing large acidophilic zymogen granules, are arranged in acini. These cells, representing pancreas, are found in association with typical ducts and islets of Langerhans.

*CASE 7.—Clinical Course.*—A 36 year old white soldier complained of slight dyspnea, which had been present for many years. A roentgenogram of the chest revealed a mass in the upper part of the anterior mediastinum, to the left of the



Figure 8  
*(See legend on opposite page)*

heart, near the pulmonary artery (fig. 10). Within two months pain developed in the left shoulder, and the patient had severe dyspnea, cough and fever. These symptoms subsided only to recur after another lapse of three months. At this time another roentgenogram of the chest showed considerable increase in the size of the mediastinal mass. The tumor was removed surgically.

*Gross Examination.*—The specimen was a cyst measuring 5.5 by 6 by 5 cm. The external surface was smooth and glistening except for several irregular hemorrhagic granular areas where the tumor was adherent to the pleura. The cyst was filled with hair and sebaceous material. The lining was smooth except for several polypoid masses, the largest of which measured 2 by 2.5 cm. The cyst was covered by "skin" having the texture of orange peel and bearing many fine lanugo hairs (fig. 9B). On section this polypoid structure was seen to consist almost solely of adipose tissue.

*Microscopic Examination.*—(a) Ectodermal Derivatives: The surface of the polypoid mass was covered by well differentiated skin (fig. 9D). The sebaceous and sweat glands were well developed, as were also the hair follicles. Large bundles of smooth muscle ran obliquely and parallel to the surface; often they were attached to the connective tissue sheath of the hair follicles as arrectores pilorum.

(b) Mesodermal Derivatives: Prominent in this group was the adipose tissue which made up the bulk of the polypoid masses noted grossly. The smooth muscle of the skin contributed to the well differentiated appearance of the structure. Smooth muscle was also found about spaces lined by ciliated columnar and mucous epithelium. In the latter connection it was arranged in both longitudinal and circular layers. A large mass of smooth muscle contained several cystic spaces lined by cuboidal epithelium. This structure resembled the normal prostate. Lymphoid tissue was often associated with both the mucous and the ciliated columnar epithelium; islands of hyaline cartilage, with the latter.

(c) Entodermal Derivatives: Irregular, often elongated and elaborately branching spaces were lined by ciliated columnar epithelium and occasional goblet cells. Accompanying these were mixed mucous and serous glands associated with hyaline cartilage, smooth muscle and lymphoid tissue. The whole organoid structure was a fair replica of a bronchiolar wall (fig. 9C). However, it was unlike a normal bronchus in that the ciliated epithelium was often abruptly replaced by stratified squamous cells. An epithelium consisting of goblet cells and accompanied by circular and longitudinal bundles of smooth muscle and lymphoid tissue closely resembled the wall of the appendix or the large intestine. Most prominent in these sections, however, were masses of well differentiated pancreas consisting of acini, islets of Langerhans and ducts.

#### EXPLANATION OF FIGURE 8

(Case 6). A, endochondral bone formation. Lamellar bone has been laid down about advancing capillaries, which are invading degenerating cartilage cells; the latter have taken up an axial position.  $\times 230$ .

B, bone lamellas showing osteocytes within the lacunas. Canaliculi radiate from the lacunas. Bodian's silver-aniline blue stain;  $\times 500$ .

C, membranous bone formation. The lamellas of bone are lined by osteoblasts, some of which have been incarcerated within the developing bone as osteocytes.  $\times 280$ .

D, interlacing bundles of smooth muscle resembling a leiomyoma in appearance. Bodian's silver-aniline blue stain;  $\times 230$ .

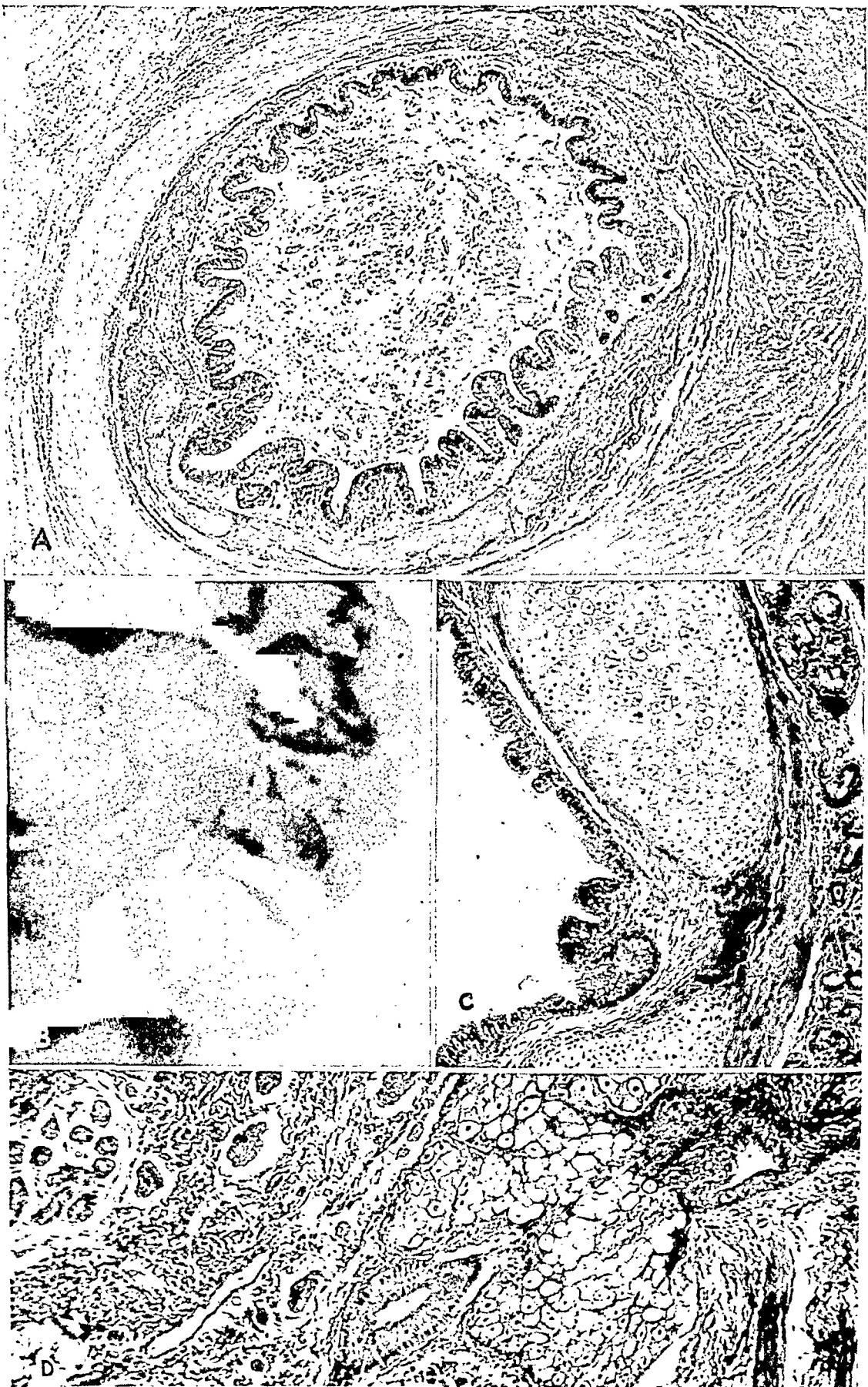


Figure 9  
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**CASE 8.—Clinical Course.**—A roentgenogram of the chest, made as part of a routine physical examination of a 20 year old white soldier, revealed in the anterior mediastinum a mass showing atypical calcification (fig. 11). There was no history of any symptoms referable to the lesion of the chest. Physical examination gave wholly negative results. At operation a cystic tumor was found "deep" in the mediastinum and removed intact. A hemopneumothorax developed but was resorbed; otherwise recovery was uneventful.

**Gross Examination.**—The specimen was a thick-walled cyst, measuring 9 by 5 by 5 cm. On section the lining surface was smooth, and the lumen was filled with sebaceous material and hair which arose from several small, partly calcified nodules.

**Microscopic Examination.**—(a) Ectodermal Derivatives: The surface of the nodules noted grossly consisted of stratified squamous epithelium, beneath which lay numerous sebaceous glands that were frequently associated with hair follicles. The sebaceous glands were very large, approaching adenomatous proportions. Several dilated sweat glands or apocrine glands were also present.

(b) Mesodermal Derivatives: Bundles of smooth muscle and groups of fat cells were scattered throughout the dense, hyalinized connective tissue which formed the supporting stroma of the cyst wall. In several regions there were irregular spaces, probably lymph channels, that closely resembled those seen in lymphangioma. Many of the spaces contained numbers of large macrophages having foamy cytoplasm. Collections of lymphoid tissue were frequently seen close by.

(c) Entodermal Derivatives: Organoid alimentary structures lined by tall columnar mucous epithelium and surrounded by well differentiated layers of smooth muscle were prominent.

**CASE 9.—Clinical Course.**—A 23 year old white woman, wife of a soldier, had had a "leaking heart" since the age of 6 years. She had been a sickly child, unable to take exercise because of shortness of breath. Recurring hemoptysis became more frequent and severe. On admission to the hospital the patient was underweight and pale, with pronounced clubbing of fingers and toes. The pulse was rapid and the heart enlarged, and a diastolic murmur was heard at the apex. The cardiac signs disappeared on rest and symptomatic treatment. Roentgenograms of the chest showed a mass in the middle lobe of the right lung, in which teeth and calcium deposits were clearly visible.

#### EXPLANATION OF FIGURE 9

A, organoid structure resembling a portion of the large intestine (case 6). The mucosa is lined by goblet cells. A muscularis mucosae and lymphoid tissue, however, are absent.  $\times 70$ .

B, cystic teratoma (case 7) which has been opened and its contents of hair and sebaceous material removed. A sessile polyp, 2 by 2.5 cm., projects into the cavity. The surface of the polyp has the texture of orange peel and bears many delicate hairs.

C, portion of the wall of an organoid respiratory structure or "bronchus" (case 7). The lumen is lined by ciliated columnar epithelium. This is separated from the adjacent hyaline cartilage by a narrow band of connective tissue. Below the cartilage are numerous mucous glands.  $\times 100$ .

D, well differentiated skin from the polyp shown in B (case 7). A hair follicle, a hair sheath and an accompanying sebaceous gland are clearly shown, cut slightly off center. At the right are the oblique smooth muscle bundles of the arrectores pilorum. At the left margin is a coiled sweat gland.  $\times 120$ .

At operation the middle lobe was found to be atelectatic and adherent to the thoracic wall. It had been displaced by a large cystic tumor which apparently arose in the anterior mediastinum and was intimately united with the pericardium. The neoplasm and the right middle lobe were removed; recovery was uneventful.

*Gross Examination.*—The tumor was a well encapsulated cystic mass weighing 450 Gm. and measuring 15 cm. in diameter (fig. 12A). The cyst wall averaged 2 mm. in thickness; on its inner surface it bore several papillary projections from which arose many long hairs. The largest of the papillae was broadly sessile,

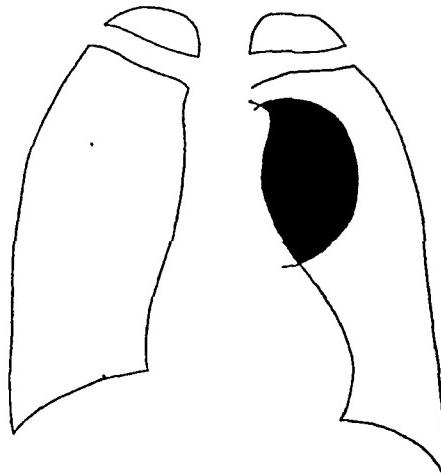


Fig. 10 (case 7).—Diagram of the roentgen shadow in the upper part of the anterior mediastinum.

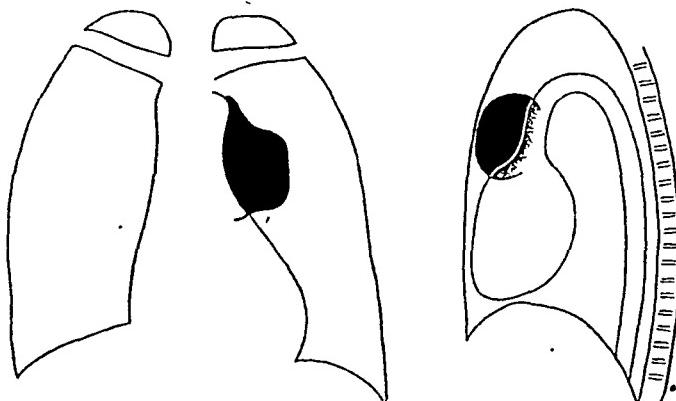


Fig. 11 (case 8).—Diagram of the roentgen shadow of a mass in the anterior mediastinum.

almost spherical in shape, and 4 cm. in diameter. On section it consisted primarily of adipose tissue. The skin that covered the papillae had a coarse "orange peel" texture. Attached to a portion of the cyst wall was an irregular piece of bone (4 by 5 by 2 cm.) bearing three visible teeth. The cyst cavity was filled with densely packed hair matted together with sebaceous material.

*Microscopic Examination.*—(a) Ectodermal Derivatives: The cyst wall, which contained remnants of the thymus gland, was composed of dense bundles of connective tissue and was lined by stratified squamous epithelium. The epithelium and underlying connective tissue had the appearance of true skin, containing well

differentiated sebaceous glands, usually associated with a hair follicle. Dilated sweat glands, often resembling apocrine glands, were likewise found. The teeth noted grossly had a histologic structure identical with that of normal teeth (fig. 12 C). The columnar odontoblasts were clearly visible at the base (fig. 12 B). The characteristic lamellar structure of the bone surrounding normal teeth was also present. Abnormal, however, was the finding of mixed mucous and serous salivary glands and ciliated columnar epithelium in intimate relation with the alveolar bone.

(b) Mesodermal Derivatives: Narrow bundles of smooth muscle, oblique to the surface of the epidermis, were frequently attached to the connective tissue sheath of the hair follicles, closely resembling normal arrectores pilorum. Smooth muscle, arranged in circular parallel bundles, was also seen, surrounding spaces lined by mucous or ciliated columnar epithelium. Elsewhere were irregular masses of smooth muscle with no apparent relation to other structures. Bone was present only in association with teeth. In some areas haversian canals with concentrically arranged lacunas were prominent. Between the bone trabeculae lay hemopoietic tissue identical with that found in normal active marrow. Hyaline cartilage was widely scattered throughout all sections, usually closely associated with ciliated columnar ("bronchial") epithelium. Occasionally, however, no such relation could be demonstrated. Lymphoid tissue, frequently containing well differentiated germinal centers, was often observed lying immediately beneath the columnar epithelium. Patches of adipose tissue were numerous and usually were surrounded by bundles of collagenous connective tissue. Blood vessels with well developed walls were abundant.

(c) Entodermal Derivatives: Prominent in all sections were small cystic spaces lined by squamous, mucous or ciliated columnar epithelium. Many of the cysts were lined wholly or predominantly by one of these three types of epithelium. In such instances, particularly in the presence of smooth muscle and lymphoid tissue, the resultant organoid structures resembled bronchi and intestine. Other cysts bore all three varieties in almost equal proportions. The squamous epithelium, however, was probably a metaplastic derivative of the columnar type. In one area, associated with mucous ("intestinal") epithelium, were numerous acini, the cells of which contained large eosinophilic zymogen granules. Nearby were spaces lined by tall columnar epithelium similar to that normally observed lining large excretory ducts. Despite the absence of islets of Langerhans, this tissue was histologically identical with pancreas.

**CASE 10.—Clinical Course.**—A 22 year old white soldier had a dry hacking cough for two years. It was accompanied by pain in the chest which gradually became more pronounced and was associated with dyspnea and palpitation after slight exertion. A definite bulge in the thoracic wall over the left precordium transmitted the heart beat but was not expansile. A roentgenogram of the chest (fig. 13) revealed great widening of the mediastinal shadow because of a mass which encroached on both the right and the left hilar lung fields. Axillary lymphadenopathy was noted, but biopsy of one of the nodes was reported to show nothing abnormal. A diagnosis of lymphoma was made, and four irradiation treatments were given, which consisted of 600 r to the anterior part of the chest and 600 r to the posterior part. Two days after the last treatment the patient became very dyspneic and cyanotic; the jugular veins were distended. A few hours later he died.

**Gross Examination.**—At autopsy an irregular tumor, measuring 19 by 30 by 15 cm., lay in the anterior part of the superior mediastinum, displacing the heart downward and backward. Although it encroached on both lungs and was broadly adherent to the pericardium, it did not invade any of these tissues. It was well

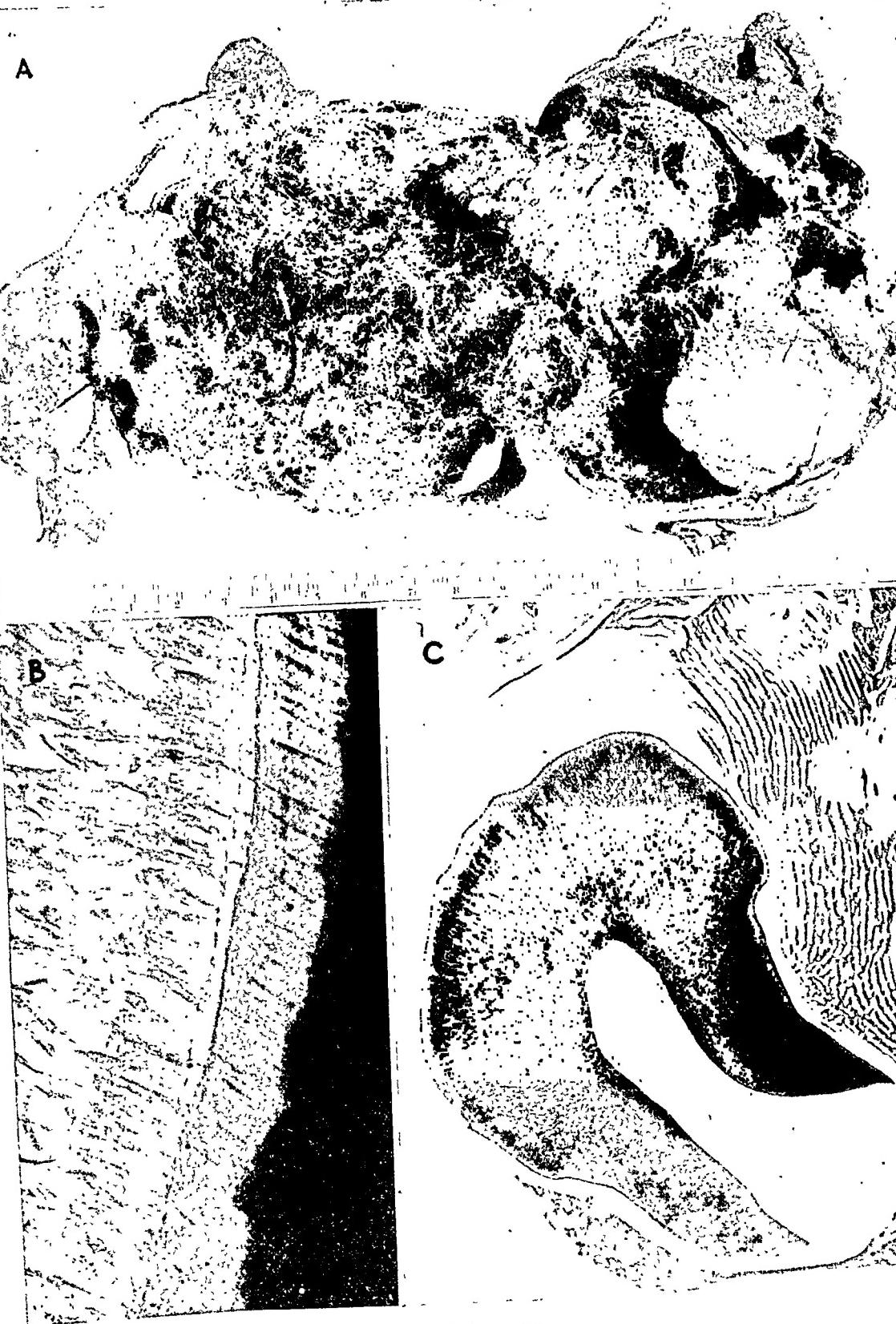


Figure 12  
*(See legend on opposite page)*

encapsulated and on section presented a varied appearance. Some areas were cystic and filled with cloudy yellow fluid; other regions were made up of glistening gray tissue containing yellow areas of calcification. Large masses of friable necrotic tissue were also present.

*Microscopic Examination.*—(a) Ectodermal Derivatives: No epidermal structures could be identified; however, nerve tissue was abundant. Large masses of neuroglia were present; the astrocytes which formed the chief cellular component were large cells with polychromatic cytoplasm suggestive of that seen in astrocytoma. Associated with the glial tissue were several small circular spaces lined by tall columnar cells closely resembling ependyma (fig. 14A). Numerous ganglion cells were seen. One of these, isolated from the rest and not associated with neuroglia, was similar to those found in cranial or spinal nerve ganglions, being surrounded by a capsule (fig. 14B). A few nests of pigmented epithelial cells were present, but whether these were of ectodermal or entodermal origin could not be determined.

(b) Mesodermal Derivatives: A loose myxomatous connective tissue formed a stroma in which were embedded large numbers of well differentiated blood vessels and many dilated capillaries. A diffuse infiltrate of neutrophils was present in many of these areas. A few islands of hyaline cartilage were scattered through the sections; these were not associated with epithelium. A single area of membranous bone formation could be identified.

(c) Entodermal Derivatives: Numerous spaces were lined by tall columnar epithelial cells having a clear cytoplasm and nuclei which were directed toward the lumen rather than toward the surrounding connective tissue (fig. 14C). Ciliated or clearly glandular epithelium could not be found.

#### GROUP II. MALIGNANT TERATOMA

Death occurred in all 6 patients with cancerous teratoma, the average duration of life after the onset of symptoms being five and one-half months. The initial symptoms were similar to those of benign teratoma and consisted of pain in the chest, cough and dyspnea. In the terminal stages, compression of the large veins in the mediastinum frequently led to impairment of venous return, particularly from the head. One patient (case 14) suffered complete motor and sensory paralysis below the level of the twelfth thoracic vertebra due to metastases in that region. In 2 instances no clinical diagnosis was obtainable from the records, although the use of irradiation therapy in one suggests

#### EXPLANATION OF FIGURE 12

(Case 9). A, cystic teratoma, which has been opened; it weighed 450 Gm. and was filled with hair and sebaceous material. On the inner surface are shown several large papillary projections covered by coarse skin from which grow long hairs. (Seven teeth arose from a piece of bone embedded in the wall; this is not shown in the figure.)

B, dentinal margin of the tooth of B. From left to right the figure shows the following layers: the cellular pulp, the relatively cell-free clear zone of Weil, the odontoblast layer, the poorly calcified predentin and more heavily calcified dentin.  $\times 400$ .

C, tooth partly embedded in laminated cancellous bone. The enamel crown was dissolved during the preparation of the section. The radial arrangement of the dentinal tubules is clearly shown. The pulp cavity, which appears almost empty in the figure, was filled by loose connective tissue.  $\times 6$ .

that a lymphoblastoma was suspected. In another case, the lesion was interpreted as a benign teratoma, and in the remaining 3 cases, as a lymphoblastoma.

The youngest patient was 19 years of age; the oldest, 29 years old; the average age was 24 years, the same as for the benign teratoma group.

Cancerous change is thought to be contemporary with rapid increase in size of the benign teratoma in late adolescence and early maturity. This was well shown by Rusby,<sup>9</sup> who found that of 174 patients whose cases are recorded in the literature, 132 experienced symptoms first while between 10 and 40 years of age and 68 during the decade of 20 to 29 years. A satisfactory explanation of the remarkable growth of mediastinal teratoma in the young adult has not been found, although various hormonal factors have been called into account. If the

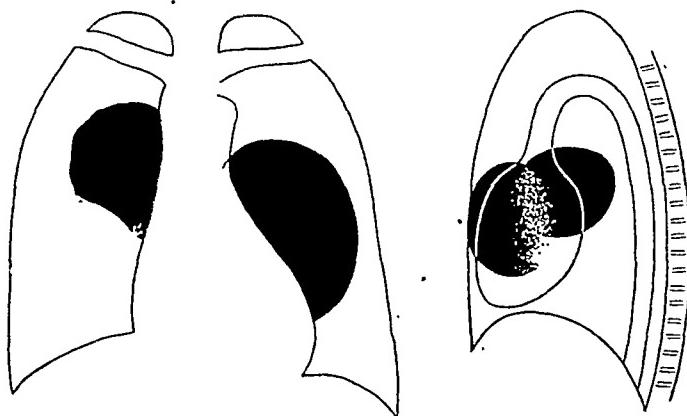


Fig. 13 (case 10).—Diagram of the widened mediastinal shadow.

mediastinal teratoma is closely linked with the thymus, as I shall later attempt to show it is, the findings of Dearth<sup>10</sup> on the development of the thymus in the cat are of interest. This author showed that in the 18 week old kitten, in which fat replacement of the thymic parenchyma is already well under way, corresponding to the condition of the thymus in the older adolescent or the young adult human, the thymus is decidedly more vascular than in preceding or subsequent stages. If this increased blood supply is also brought to the intimately associated teratoma, it may provide a partial explanation of the rapid growth of this tumor at that time.

**CASE 11.—Clinical Course.**—A 20 year old white soldier contracted a slight head cold in October 1940 and by Nov. 4, 1940 had a cough and some pain in the chest. Five days later he was admitted to the hospital with a diagnosis of lobar pneumonia, lower lobe of the right lung. He was given sulfapyridine after a roentgenogram showed consolidation of the entire right side of the chest (fig. 15).

10. Dearth, O. A.: Am. J. Anat. 41:321, 1928.

Thoracentesis yielded 200 cc. of straw-colored fluid, which gave no growth on culture. The patient showed no improvement; he had a rather unproductive cough and fever. There was a loss of 20 pounds in weight during the six months before death. Bronchoscopy showed compression of the right main bronchus, interpreted as extrabronchial in origin. As the patient was in no physical condition for operation, irradiation therapy was resorted to, but it failed to decrease the size of the mass. The temperature and the pulse rate remained elevated, and the patient died with evidence of circulatory failure four months after the onset of symptoms.

*Gross Examination.*—At autopsy the superior mediastinum was filled by a smooth, slightly lobulated tumor that displaced the heart to the left and partly compressed the left lung. The right lung was completely atelectatic, since most of the right side of the thorax was occupied by the neoplasm. The tumor weighed 2,300 Gm., was roughly spherical and had a mean diameter of 20 cm. On section, connective tissue trabeculae extended from the capsule into the parenchyma, dividing it into lobules of varying size. Most of the tissue had a fleshy appearance and contained scattered mucin-filled cysts measuring up to 1 cm. in diameter.

The liver weighed 2,000 Gm. and showed the characteristic changes of chronic passive hyperemia. A spherical, partly necrotic tumor, 10 cm. in diameter, was found within the organ.

*Microscopic Examination.*—(a) Ectodermal Derivatives: No structures traceable to this germ layer could be identified.

(b) Mesodermal Derivatives: The connective tissue stroma was loose, almost myxomatous in character. Small areas of hemorrhage and necrosis were scattered throughout the sections; these were surrounded by large numbers of neutrophils. In one region were large bundles of striated muscle (fig. 16C); these were branched and had centrally placed nuclei; hence they may be identified as cardiac muscle. The blood vessels had poorly differentiated walls, which often consisted solely of a single layer of endothelial cells.

(c) Endodermal Derivatives: Many spaces were lined by ciliated columnar epithelium, but nowhere did this form a part of organoid respiratory structures. The ciliated epithelium was often abruptly replaced by tall goblet cells (fig. 16A), and the cyst was filled with mucin. Pleomorphic epithelial cells with one or more bizarre nuclei were present in all sections. They were found as sheets, isolated cells or cells forming irregular abortive acini. These cells were clearly cancerous and may be classified as adenocarcinoma (fig. 16B), but the differentiated cell from which they were derived is unknown.

The hepatic metastasis consisted of loose connective tissue, cardiac muscle and adenocarcinoma (fig. 16D).

**CASE 12.—Clinical Course.**—A 21 year old white man was admitted to the hospital with a history of having had cough and bloody mucoid sputum for the past three days, and chills and fever for one day. He was treated for pneumonia, improved under the regimen and was discharged. A month later he returned, complaining of cough, bloody sputum and pain in the chest; he was orthopneic and dehydrated. A roentgenogram of the chest disclosed a small effusion on the left side, associated with pneumothorax. A mass in the mediastinum infiltrated or displaced the hilar regions of both lungs (fig. 17). A diagnosis of lymphoblastoma was made and irradiation therapy administered. He died three weeks after his second admission, two months after the onset of symptoms.

*Gross Examination.*—At autopsy a large tumor, forming two rather discrete masses, was present in the anterior mediastinum. One mass measured 18 by 14 by 9 cm., was firm and nodular and invaded the right lung. The other mass, which

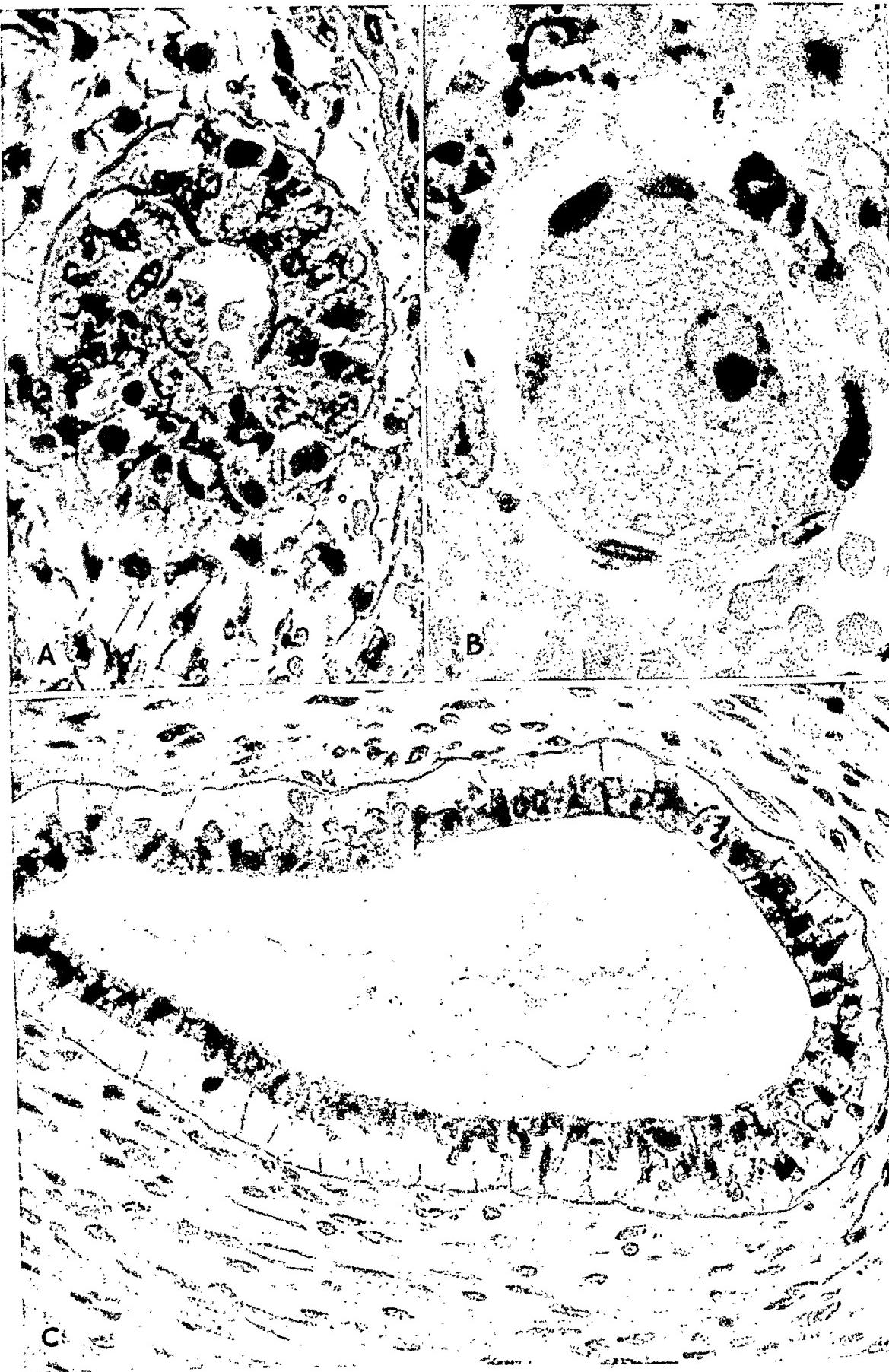


Figure 14

(See legend on opposite page)

measured 20 by 12 by 8 cm., was soft, friable and reddish brown. The bronchi of both lungs were clear. No distant metastases were found.

*Microscopic Examination.*—(a) Ectodermal Derivatives: Irregular small cysts were lined by stratified squamous epithelium. In none of the sections were there any organoid cutaneous structures. A few scattered collections of pigmented epithelium were present.

(b) Mesodermal Derivatives: The connective tissue stroma was reticulated; many of the cells were stellate; bundles of dense collagenous fibers were uncommon. Blood vessels were not numerous and, when found, had poorly developed walls. Several areas of necrosis were present; about their margins were infiltrates of degenerating neutrophils.

(c) Endodermal Derivatives: Alimentary and respiratory organoid structures were absent. Groups of acinous pancreatic tissue associated with islets of Langerhans and dilated ducts were numerous. Irregular masses of deeply staining epithelial cells with large vesicular nuclei were scattered throughout the sections. Histologically they had the character of cancerous epithelial cells, which in some regions were grouped into poorly formed acini; elsewhere they were wholly undifferentiated. The appearance of these cells and their relation to the pancreatic tissue suggested that the latter may have undergone cancerous change.

**CASE 13.—Clinical Course.**—A 28 year old soldier was apparently well until four months before death. At that time he began to have a nonproductive cough, dyspnea, pain in the left side of the chest and an evening rise of temperature. A month after the onset of symptoms a roentgenogram of the chest revealed a mass in the mediastinum that extended into the hilus of the left lung (fig. 18). At that time a diagnosis of Hodgkin's disease was made, although mediastinal teratoma and carcinoma of the lung were also considered. The patient received irradiation therapy, 1,000 r to the anterior mediastinum and 1,000 r to the posterior mediastinum, without apparent benefit. Subsequently, another course totaling 600 r to the posterior and 2,100 r to the anterior mediastinum likewise failed to reduce the size of the lesion. All symptoms became progressively more severe, the liver became palpable, and shortly before death petechiae and ecchymoses were prominent and diffuse.

*Gross Examination.*—The right pleural cavity contained 500 cc. of blood-tinged fluid; the left was wholly obliterated by a tumor that had pushed the mediastinal structures into the right side of the chest. Although the superior mediastinum was filled with a mass of tumor tissue, the aorta and the esophagus were free. Except for a small portion of the apex, the left lung was completely replaced by a soft, necrotic, yellow-pink, hemorrhagic neoplasm that apparently arose in the mediastinum. On section several cystic spaces containing liquefied necrotic tissue were observed. The medial half of the upper lobe of the right lung was also invaded by the tumor. Metastatic nodules were present in the liver.

#### EXPLANATION OF FIGURE 14

(Case 10). *A*, ependyma-like structure surrounded by neuroglia.  $\times 700$ .

*B*, ganglion cell similar to those normally found in the spinal and cranial nerve ganglia. The axon is seen leaving the cell at the top of the figure. Surrounding the cell body is a layer of sheath cells. The pale-staining polygonal cells are erythrocytes.  $\times 1,360$ .

*C*, small cyst lined by columnar epithelium. The position of the nuclei is remarkable, being near the free surfaces of the cells rather than at their bases. The basal parts of the cells contain mucinous material.  $\times 500$ .

*Microscopic Examination.*—(a) Ectodermal Derivatives: Epidermis, cutaneous appendages or nerve tissue could not be identified.

(b) Mesodermal Derivatives: A loose reticular network of stellate cells formed the connective tissue stroma of the tumor. Large numbers of irregular vascular spaces were present, the walls composed of a single layer of endothelium. No cartilage or bone was seen.

(c) Entodermal Derivatives: Irregular, poorly differentiated alveoli alternated with well formed acini lined by tall columnar epithelium, the nuclei of which were characteristically near the free surfaces rather than at the basal parts of the cells. In some regions the cells lining the alveoli were clearly cancerous and related to the large cells with polychromatic cytoplasm that were scattered throughout the sections (fig. 19A). The metastasis in the liver was structurally identical with the primary lesion.

**CASE 14.—Clinical Course.**—A 29 year old white soldier had no symptoms until six months before death, at which time he noted pain in the left side of the chest, radiating into the left shoulder and arm. Three weeks later dyspnea and vertigo

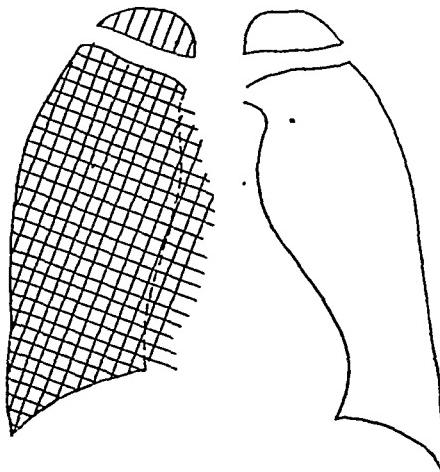


Fig. 15 (case 11).—Diagram of the roentgen appearance of the chest.

developed on exertion, symptoms which continued to occur with increasing severity until the time of death. Roentgenograms of the chest a month after the onset of symptoms showed prominence of the pulmonary artery, leading to a tentative diagnosis of aneurysm of the pulmonary artery. On the basis of subsequent roentgenologic studies, the diagnosis was changed to one of mediastinal or pulmonary tumor, probably lymphoblastoma (fig. 20). During one brief period the patient expectorated bloody sputum. A month before death there was sudden onset of excruciating pain low in the back with complete paralysis of the lower extremities. Bronchoscopy, done once, revealed narrowing of the left main bronchus but no recognizable bulging of tumor into it.

*Gross Examination.*—At autopsy the anterior mediastinum contained a mass, 15 by 9 by 7 cm., weighing 610 Gm. It was adherent to the pericardium and the upper lobe of the left lung but did not arise from any portion of the bronchial tree or the lung parenchyma. The cut surface of the tumor showed necrotic, gray, soft tissue with intermingling fibrous strands. The twelfth thoracic vertebral body was collapsed because of tumor growth similar to that in the mediastinum.

*Microscopic Examination.*—(a) Ectodermal Derivatives: Skin or nerve tissue could not be identified.

(b) Mesodermal Derivatives: The connective tissue stroma was loose, myxomatous, with only occasional bundles of collagenous fibers. Adipose tissue formed small islands that were scattered throughout the sections. There were many blood vascular spaces with walls consisting of a single layer of endothelium. Islets of developing hyaline cartilage and membranous bone were found in two sections (fig. 19B).

(c) Entodermal Derivatives: Throughout the parenchyma were irregular alveolar spaces formed by neoplastic epithelial cells, which elsewhere were grouped in amorphous masses. The cells were pleomorphic, with abundant cytoplasm and one or more large, often bizarre nuclei.

CASE 15.—*Clinical Course.*—A 19 year old white soldier was apparently well until three months before his death, at which time he experienced a dull aching pain over the left side of the chest, which did not radiate, was intermittent and was more noticeable on inspiration. About six weeks after the onset of symptoms he was admitted to the hospital with a diagnosis of pleurisy. Physical examination gave essentially negative results. A roentgenogram of the chest revealed a tumor that was interpreted as "benign dermoid cyst of the anterior superior mediastinum" (fig. 21). A therapeutic test dose of 1,800 r was given; no response was noted, and irradiation therapy was discontinued. During the last week of life the patient became drowsy and had a temperature of 101 F.

*Gross Examination.*—At autopsy the anterior mediastinum was occupied by a fluctuant tumor, 20 by 22 cm. It was adherent to both lungs and had infiltrated the inferior medial portion of the upper lobe of the left lung. Each pleural cavity contained approximately 1,500 cc. of blood-streaked fluid. The neoplasm surrounded the heart, completely encircling and constricting the great vessels. It filled the pericardial sac and infiltrated the epicardium. Large necrotic areas, as well as cysts filled with a mucoid substance, were encountered on section of the tumor.

*Microscopic Examination.*—(a) Ectodermal Derivatives: No structures belonging to this germ layer could be identified with certainty; atypical neuroglia might be represented. Several cystic spaces were lined by squamous epithelium, but this may be interpreted as metaplasia of mucous epithelium.

(b) Mesodermal Derivatives: Large numbers of poorly differentiated blood vessels were present in all sections. In the many necrotic areas the tissue was frequently flooded with erythrocytes that had escaped from the thin-walled sinuses. Polymorphonuclear leukocytes often surrounded these regions in large numbers. Several islands of young, well differentiated hyaline cartilage were present (fig. 19C).

(c) Entodermal Derivatives: In all sections of the tumor there were large pleomorphic epithelial cells with one or more bizarre nuclei. Occasionally these cancerous cells were intimately associated with alveolar structures, the columnar epithelial cells of which showed early neoplastic changes (fig. 19D). Irregular spaces lined by cuboidal epithelium were embedded in a loose connective tissue. Several cysts lined by goblet cells could also be identified.

CASE 16.—*Clinical Course.*—A 29 year old white soldier was apparently well until two months before death, when he had a severe cold associated with a nonproductive cough, pain in the right side of the chest and chills. Hospitalization was not necessary, and the patient apparently recovered completely. While on board ship en route to England, he became dyspneic and was confined to the ship's hospital for three days. A slight elevation of temperature was noted during his hospitalization, but he felt improved at the time of his release, although still



Figure 16  
*(See legend on opposite page)*

somewhat dyspneic. On admission to an army hospital in England a week before he died, the right side of the patient's face was swollen and the veins of the neck were distended, most markedly on the right. The trachea and the heart were displaced to the left; the right side of the chest was dull to percussion, and there was bulging of the intercostal spaces. Thoracentesis yielded 500 cc. of bloody fluid. Thereafter until death, seven days later, dyspnea became more severe and was relieved only by periodic thoracic taps. On the day of death the head and neck were cyanotic, the external jugular veins were distended, and the right arm was edematous. The patient died during a venesection performed to relieve the embarrassment of the right side of the heart.

*Gross Examination.*—At autopsy the right thoracic cavity was filled with 3,500 cc. of bloody fluid. After removal of the fluid a large neoplasm was seen that occupied the upper two thirds of the right side of the chest and compressed the right lung. The tumor measured 16 by 11 by 8 cm., was situated just anterior to the bifurcation of the trachea and occupied most of the superior and middle portions of the anterior mediastinum. The friable mass was soft, red-gray, and well encapsulated for most of its circumference. Scattered throughout the parenchyma were gelatinous areas of degeneration. The right main stem bronchus and its branches were opened, but tumor tissue could not be demonstrated. The parietal pleura of the right side of the thorax was studded with tumor metastases. The right hilar lymph nodes and the diaphragm were likewise invaded by the neoplasm.

*Microscopic Examination.*—The over-all picture was one of loose cellular connective tissue, possibly cancerous, through which were scattered poorly formed acini and solid masses of neoplastic epithelial cells. The metastatic nodules on the pleura had a similar structure.

(a) Ectodermal Derivatives: No organoid cutaneous structures could be identified: squamous epithelium was absent. The only ectodermal derivatives that could be identified were occasional areas composed of neuroglia. Within the latter were scattered rosettes which resembled ependyma, but whether they were cannot be established with certainty.

(b) Mesodermal Derivatives: The connective tissue stroma varied in cellularity, but on the whole the nuclei were abundant and variable in size, with irregular shapes and staining properties similar to those seen in fibrosarcoma. The cells were stellate, and in the gelatinous regions noted grossly they resembled those of myxoma. Irregular and dilated lymph channels lent a lymphangiomatous character to some areas.

(c) Entodermal Derivatives: The epithelium which was sufficiently well differentiated to warrant classification resembled that of the normal intestine. In some regions the cells were grouped to form ducts and acini. In these structures the nuclei were frequently arranged along the free surfaces instead of at the

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#### EXPLANATION OF FIGURE 16

(Case 11). A, lining epithelium of a small cyst. There is an abrupt transition from tall columnar mucous cells to ciliated columnar epithelium.  $\times 500$ .

B, loosely arranged cancerous epithelial cells forming no particular pattern.  $\times 500$ .

C, striated muscle; in two of the fibers a centrally placed nucleus can be recognized, indicating that the muscle is of the cardiac type.  $\times 1,360$ .

D, branched muscle fibers and cancerous epithelial cells in a metastatic nodule of the liver.  $\times 500$ .

bases of the cells (fig. 19E). Much of the epithelium was frankly cancerous, forming solid clumps of cells, syncytial masses or abortive acini and ducts (fig. 19F).

#### HISTOLOGIC COMPARISON OF THE BENIGN AND THE CANCEROUS TYPE OF MEDIASTINAL TERATOMA

Benign and cancerous specimens of teratoma differ greatly in microscopic appearance. Of primary importance is the occurrence of well

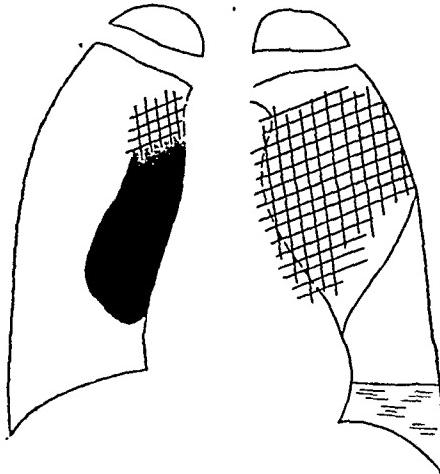


Fig. 17 (case 12).—Diagram of the roentgen shadow of a mass in the mediastinum.

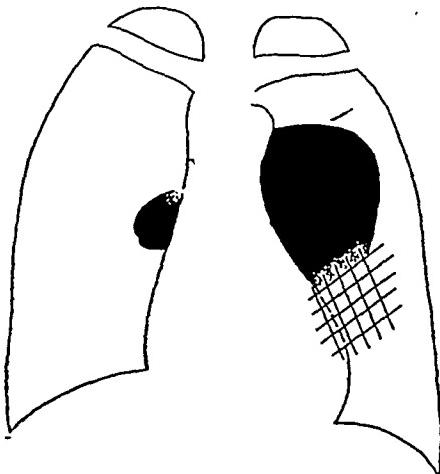


Fig. 18 (case 13).—Diagram of the roentgen shadow of a mass in the mediastinum.

differentiated structures in the benign group, few such structures being observed in the cancerous group. Recognizable ectodermal derivatives are almost completely absent in the cancerous group, whereas they are prominent in the benign. The connective tissue stroma of cancerous teratoma is usually loosely arranged, whereas that of benign teratoma is dense and of adult type. In all 6 specimens of cancerous teratoma

large areas were histologically adenocarcinoma; in none of the specimens of benign teratoma did any region resemble cancer.

#### STRUCTURAL COMPONENTS OF MEDIASTINAL TERATOMA AND THEIR RELATIONSHIPS

As in teratoma of other regions the structures that compose mediastinal teratoma are intermingled without apparent order. This intermingling of well differentiated tissues and organoid structures in the benign type of teratoma has repeatedly led investigators to the assumption that this type represents a malformed fetus. The problem of its genesis will be considered in the following section. Here I am concerned only with showing that experimental embryology can account for the mixture of tissues which the tumor contains. What imparts the neoplastic character to teratoma is as yet wholly unknown.

The formative interrelationship of tissues was recognized long before the concept of the organizer had been elaborated. Thus, in 1903 Beneke<sup>11</sup> pointed out that the manner and the rate of differentiation of connective tissue are dependent on the type and the character of the overlying epithelium. He emphasized the importance of Wilhelm Roux's *Entwicklungsmechanik* for the study of tissue relations in neoplasia. This concept was applied to teratoma by Budde,<sup>12</sup> who insisted that it is not permissible to identify epithelium with reference to origin on the basis of morphologic aspects alone; rather, the effect which it exercises on the underlying connective tissue must be taken into account. For example, epithelium of the intestinal type is likely to be accompanied by lymphoid tissue and smooth muscle; whereas epithelium of the respiratory type is characteristically associated with cartilage. More recently Willis<sup>13</sup> and Nicholson<sup>14</sup> have studied the morphology of teratoma from this point of view.

Under certain conditions any tissue of an embryo may act to induce in the adjacent tissue the formation of structures other than those normal to the region. As pointed out in the introduction, the agent responsible for the change may be liberated by killing the cells in boiling water. However, in the living organism less drastic processes must suffice to liberate the evocative substance. Needham<sup>15</sup> emphasized the fact that under abnormal conditions the liberated evocator may induce the formation of organoid structures but never of complete organs. Hence the

11. Beneke, R.: Zur Histologie der foetalen Mamma und der gutartigen Mammatumoren, in Pathologisch-anatomische Arbeiten Herrn geh. Medicinalrath Johannes Orth zur Feier seines 25jährigen Professoren-Jubiläums, Berlin, A. Hirschwald, 1903, p. 570.

12. Budde, M.: Beitr. z. path. Anat. u. z. allg. Path. **68**:512, 1921.

13. Willis, R. A.: J. Path. & Bact. **40**:1, 1935.

14. Nicholson, G. W.: Guy's Hosp. Rep. **88**:263, 1938.

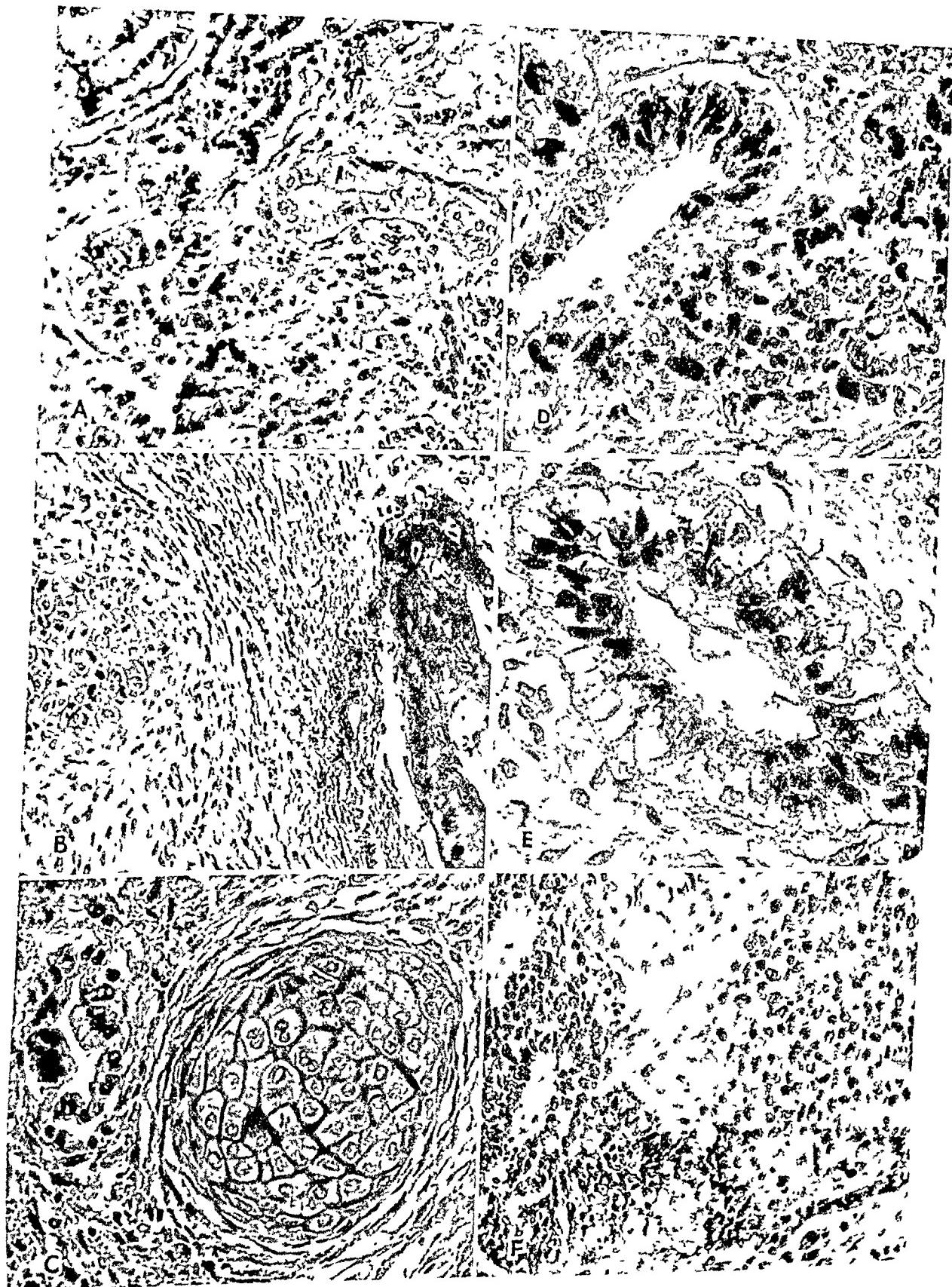


Figure 19

(See legend on opposite page)

term "evocator" rather than "organizer" in this connection. On this basis the weird hodgepodge of tissues in teratoma becomes understandable. Moreover, the conditions that bring about the liberation of the evocator may increase the capacity of the tissue to differentiate, with the formation of more varied structures during normal embryogenesis.<sup>15</sup>

*Ectodermal Derivatives.*—Nerve tissue, skin and teeth were found in all specimens of benign teratoma but in none of those of cancerous teratoma except 1 in which neuroglia and ependyma were noted. In mediastinal teratoma nerve tissue was not as prominent as it is in ovarian teratoma; only in 2 cases (6 and 10) could it be identified with certainty. In case 6 well differentiated ganglion cells were associated with neuroglia (fig. 5C). In case 10 neuroglia and ependyma were found in association with each other. In this case a single large ganglion cell of the type characteristic of cranial or spinal nerve ganglions (fig. 14B) was discovered in a vascular area; neuroglia was absent.

Skin and its appendages were present in most of the specimens of benign teratoma and were remarkably well formed. Hair follicles preserved their normal, slightly oblique position relative to the free surface and were always accompanied by well developed sebaceous glands. These glands were usually hyperplastic, frequently assuming an almost adenomatous character (fig. 2A). Occasionally, groups of sebaceous glands were found unassociated with other skin derivatives. Sweat glands, often of the apocrine type, lay near the sebaceous glands; they deviated from the normal in having a more superficial position in the corium. Smooth muscles, which closely resembled arrectores pilorum, were occasionally encountered (fig. 9D).

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15. Rudnick, D.: J. Exper. Zool. **71**:83, 1935.

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#### EXPLANATION OF FIGURE 19

*A*, cancerous epithelial cells appearing as irregular clumps or poorly formed alveoli (case 13).  $\times 211$ .

*B*, immature hyaline cartilage and bone lamella in loose connective tissue (case 14). Loose connective tissue is characteristic of this series of cases of cancerous teratoma.  $\times 160.5$ .

*C*, island of young cartilage in a loose connective tissue stroma (case 15). Two irregular alveoli of cancerous epithelial cells are shown in the left half of the figure.  $\times 275$ .

*D*, cancerous epithelial cells forming a pseudoglandular acinus (case 15); many cells have separated and infiltrated the surrounding connective tissue.  $\times 458$ .

*E*, acinous arrangement of cells that superficially resemble goblet cells (case 16). The nuclei are at the free ends rather than at the bases of the cells. The irregularity of this structure as it occurs in a cancerous teratoma is apparent when it is compared with a similar one found in a benign teratoma (fig. 14C). The surrounding connective tissue is loosely arranged and cancerous in appearance.  $\times 458$ .

*F* (case 16), another area from the same section as *E*, showing frankly cancerous epithelium arranged as solid clumps of cells and malformed acini and ducts.  $\times 211$ .

Teeth were found only in case 9; the histologic relationships were entirely normal (fig. 12 C). The outline of the enamel crown showed that it was well developed. The dentin exhibited the normal radial striations, due to the presence of dentinal tubules. Near the bases of the teeth the characteristic palisades of odontoblasts were clearly visible (fig. 12 B). In this connection should be cited the experiments of Huggins,

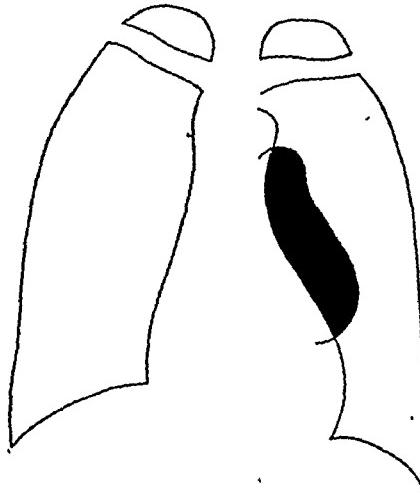


Fig. 20 (case 14).—Diagram of the roentgen shadow of a mediastinal mass.

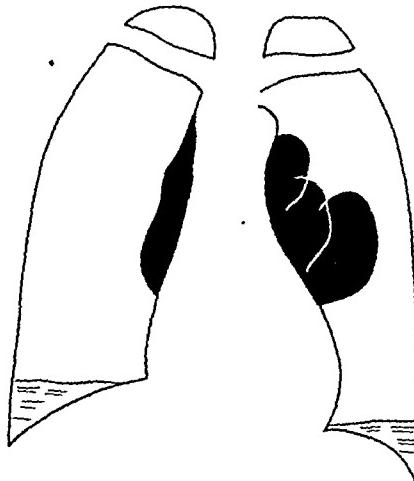


Fig. 21 (case 15).—Diagram of the roentgen shadow of a tumor adherent to both lungs.

McCarroll and Dahlberg,<sup>16</sup> who found that ameloblasts of transplanted tooth germs developed normally only when in contact with odontoblasts. If this contact was lost, the cylindric character of the ameloblasts disappeared, and they formed a stratified squamous or cuboidal epithelium.

16. Huggins, C. B.; McCarroll, H. R., and Dahlberg, A. A.: J. Exper. Med. 60:199, 1934.

*Mesodermal Derivatives.*—These were represented by the supporting tissues—connective tissue, bone, cartilage and muscle. The connective tissue proper consisted of both collagenous bundles and elastic fibers. These were arranged about organoid epithelial structures as a tunica propria; they appeared as areolar tissue beneath the "skin" and formed a diffuse supporting stroma for the tumor parenchyma. Adipose tissue was always intimately associated with the connective tissue. Bone and cartilage were often encountered. The bone was usually of the membranous type (fig. 8 *B* and *C*) and unassociated with any other differentiated structures, but in 1 case it formed the alveolus for a tooth (fig. 12 *C*) precisely as it does in a normal jaw.

The ability of certain tissues to provoke the development of bone in adjacent areas has been well demonstrated by Huggins,<sup>17</sup> who used transitional epithelium of the dog's urinary bladder as organizer and rectus sheath as substrate. Such a relation of bone to epithelium was not observed in these cases of teratoma. However, a clear relationship was seen to exist between ciliated epithelium and hyaline cartilage. Rarely, islands of cartilage were found unassociated with epithelium; usually, they formed arched plaques that followed the curve of the epithelium. As noted by Huggins for bone, a thin band of connective tissue always separated the epithelium from the cartilage, preventing direct contact of the two tissues.

Hemopoietic tissue was found only in association with cancellous bone. In 1 instance it accompanied endochondral bone formation that could scarcely be distinguished from the normal (fig. 8 *A*). Lymphoid tissue was characteristically found in the "submucosa" of organoid "intestinal" structures. Just as cartilage was usually intimately related to ciliated ("respiratory") epithelium, so lymphoid tissue most often accompanied "intestinal" epithelium (fig. 2 *D*).

Smooth muscle was most often observed as longitudinal or circular bundles in organoid alimentary structures. Occasionally it was also seen in "bronchial" walls. Oblique bundles of smooth muscle passed from the "subcutaneous" tissue to the connective tissue sheaths of the hair shafts as *arrectores pilorum* (fig. 9 *D*). In some regions masses of interlacing bundles of smooth muscle were observed that resembled leiomyoma (fig. 8 *D*) and bore no relationship to epithelial tissues. In one section of case 11 striated muscle fibers were seen (fig. 16 *C*); these were of the cardiac type, with central nuclei and branching muscle bundles. In view of the fact that smooth muscle greatly preponderated over the striated variety in these specimens, it is interesting to note that Carey<sup>18</sup> was able to transform smooth

17. Huggins, C. B.: Arch. Surg. 22:377, 1931.

18. Carey: Am. J. Anat. 29:341, 1921.

*The Structural Components and the Location of Metastases in Sixteen Cases of Mediastinal Teratoma*

Case	Ectodermal Derivatives			Mesodermal Derivatives						Entodermal Derivatives						
	Squamous Epithelium	Skin Appendages	Nerve Tissue	Connектив Tissue	Vascular Spaces	Adipose Tissue	Lymphoid Tissue	Hemopoietic Tissue	Smooth Muscle	Cartilage	Bone	Respiratory Epithelium	Intestinal Epithelium	Mucous Glands	Pancreas	Adeno-carcinoma
1	+	..	..	+	..	..	..	..	..	..	..	..	..	..	..	..
2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
3	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
4	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
5	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
6	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
7	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
8	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
9	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
432	Group I Benign teratoma			Group II Cancerous teratoma						Liver Vertebra Lymph node						
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muscle of the urinary bladder into striated muscle of the myocardial type by forcing it to undergo rhythmic contractions.

*Entodermal Derivatives.*—These include "intestinal" and "respiratory" structures and pancreas. The manner in which these epithelia reacted to supporting mesodermal structures has been discussed in the preceding section. Their interrelations were frequently intimate, and transition from one type of epithelium to the other was often abrupt (figs. 5 B and 16 A). An explanation of this intimate association is furnished by the experiments of Rudnick.<sup>19</sup> This investigator transplanted the lung primordia, including the adjacent gut wall and splanchnic mesoderm, of the 3 day chick embryo to the chorioallantoic membrane of the hen's egg. Parts of the entoderm which normally were not destined to take part in the formation of the respiratory tract showed a marked tendency to form respiratory structures. Occasionally both respiratory and intestinal epithelium lined the same tube, and one type of epithelium was frequently found to merge into the other. In another experiment Rudnick and Rawles<sup>20</sup> obtained complexes, lined by cells resembling intestinal epithelium and surrounded by smooth muscle, in grafts which originally contained no entoderm. From this they concluded that epithelia from other germ layers may not only take on a form characteristic of intestinal lining but may be associated with connective tissue and muscle to simulate alimentary structures. These experiments should serve as a warning against the too facile identification of structures found in teratoma.

One of the most surprising observations made in this group of specimens of benign teratoma was the frequency of well differentiated pancreas. Pancreas was observed as large, readily identifiable masses in 6 of the 10 specimens; in 5 of these, islets of Langerhans were associated with the acinous tissue (figs. 2 B and 5 A). Pancreatic tissue may have been seen in a seventh instance, case 2, but could not be identified with certainty. In 1 instance of cancerous teratoma (case 12) pancreas with islets was likewise present. In most instances the acinous cells, islets and ducts were so well differentiated and in such harmonious relationship that they could not be distinguished from those of normal pancreas. The high incidence of pancreas in these specimens of mediastinal teratoma as compared with its infrequent occurrence in those of teratoma of the gonads and other sites, may aid in determining the site from which teratoma takes its origin. This will be considered further in the section on the genesis of teratoma. It should, however, be noted that the literature contains few references to pancreatic tissue in mediastinal teratoma. The cases of Irene

19. Rudnick, D.: *J. Exper. Zool.* **66**:125, 1933.

20. Rudnick, D., and Rawles, M. E.: *Physiol. Zool.* **10**:381, 1937.

Gordon<sup>21</sup> and Hablützel<sup>22</sup> are the only ones listed by Rusby; another was reported by Harrington.<sup>23</sup>

#### GENESIS OF TERATOMA

For centuries teratoma has been a cause of wonder and superstition. In times past, according to Gould and Pyle,<sup>24</sup> teratoma in the male was regarded as a repetition of the process by which Eve was born of Adam. The finding of ovarian teratoma in the body of a young woman was considered as conclusive proof that she had been unchaste. With the development of embryology and teratology during the first half of the nineteenth century, cases of teratoma were classified into two main groups: (1) ovarian and testicular teratoma, which were believed to arise spontaneously from germ cells by a process of parthenogenesis, and (2) all other cases of teratoma, in which the finding was regarded as an included twin—that is to say, as a "fetus in fetu." Both of these hypotheses, now well over a century old, are still widely accepted.

*Hypothesis of Fetus in Fetu.*—In a long series of articles Nicholson carefully analyzed teratomatous specimens of the most varied origin and structure. In none was he able to find any evidence of fetiformity. In reviewing cases described by earlier investigators as instances of abortive fetus he was repeatedly able to show that the limbs, head, and "well developed organs" reported by these observers were actually the product of a too vivid imagination. In his characteristic style he wrote<sup>25</sup>: "Since it is an amiable weakness of teratology to fancy that the words similarity and identity are synonymous, we readily see in a horrid tumor a smiling babe." Willis,<sup>13</sup> in a paper that is one of the outstanding contributions to teratology, listed the following reasons for concluding that teratoma is not a distorted fetus: (1) with extremely rare and doubtful exceptions, teratoma exhibits no signs whatever of axiation, metamerie segmentation or delamination of germ layers; (2) teratoma possesses no organs or true somatic regions; (3) teratoma frequently contains a multiplicity of certain constituents, e. g., dozens of "tonsils," many separate embryonic "nervous systems"; (4) teratoma characteristically shows an anomalous absence of vital tissues; (5) abnormal tissue relationships and mixtures are frequent in specimens of teratoma.

21. Gordon, I.: Frankfurt. Ztschr. f. Path. 40:224, 1930.

22. Hablützel, C.: Schweiz. med. Wchnschr. 63:1308, 1933.

23. Harrington, S. W.: J. Thoracic Surg. 1:663, 1932.

24. Gould, G. M., and Pyle, W. L.: Anomalies and Curiosities of Medicine, Philadelphia, W. B. Saunders Company, 1897.

25. Nicholson, G. W.: Guy's Hosp. Rep. 84:389, 1934.

*Germ Cell Hypothesis.*—By demonstrating that teratoma is not a malformed fetus, the hypothesis of fetus in fetu is disposed of. But the problem of a so-called parthenogenetic development of the germ cells in the gonads is not so easily handled. The work of Jacques Loeb and of many investigators following him has conclusively proved that the vertebrate egg cell can begin development after application of stimuli other than that of sperm entry. In a long monograph Bosaeus<sup>26</sup> showed that frogs' eggs induced to develop parthenogenetically and then placed into the celomic cavity of the parent frog produced masses resembling in general structure human ovarian teratoma. More recently Greene<sup>27</sup> succeeded in transplanting rabbit embryos, either in whole or in part, to the anterior chambers of the eyes and to the testicles of rabbits or guinea pigs. He obtained growths which resembled teratoma and which could be transferred serially. On the basis of Greene's work it may be argued that Bosaeus has merely demonstrated the ability of embryonic tissue to survive and differentiate for a time under abnormal circumstances. However, some evidence favoring the germ cell hypothesis has been obtained through the recent experimental production of testicular teratoma.

In 1926 Michalowsky,<sup>28</sup> and after him Falin<sup>29</sup> and Bagg,<sup>30</sup> produced teratoma in the testis of the rooster by injecting 0.3 cc. of a 5 per cent solution of zinc chloride. Efforts to produce teratoma in extragonadal tissues by this method have failed.<sup>31</sup> The cells that give origin to teratoma have not been specifically identified; Falin has regarded them as pluripotential testicular cells. That they may well be primitive germ cells is indicated by the fact that teratoma arises only following an injection made during the spring months when spermatogenesis is most active and that teratoma cannot be induced in the immature testis. Falin expressed the belief that an important role is played by the focal necrosis resulting from the injection of the zinc compound. He considers it likely that the death and disintegration of cellular elements liberate evocators which act on testicular cells. This explanation is supported by the results which Holtfreter obtained after transplanting bits of boiled tissue into the amphibian blastocoele.<sup>6</sup>

In view of these findings it appears probable that teratoma of the testes or the ovaries arises from pluripotent germ cells normally present in these organs. A sharp distinction must be drawn, however, between

26. Bosaeus, W.: Beiträge zur Kenntnis der Genese der Ovarial-embryone, Uppsala, Almqvist & Wiksell, 1926.

27. Greene, H. S. N.: Cancer Research 3:809, 1943.

28. Michalowsky, I.: Centralbl. f. allg. Path. u. path. Anat. 38:585, 1926.

29. Falin, L. I.: Am. J. Cancer 38:199, 1940.

30. Bagg, H. J.: Am. J. Cancer 26:69, 1936.

31. Bischoff, F.; Long, M. L., and Rupp, J. J.: Am. J. Cancer 38:404, 1940.

the pluripotent precursors of the germ cells and the mature germ cells themselves. The primitive pluripotent germ cells, which eventually become egg cells or sperm, are not such as these at the time of inception of teratoma. The egg cell is not undifferentiated; rather it is a highly specialized cell which when fertilized reacts by producing an embryo—not a teratoma. Experimental parthenogenesis likewise invariably produces a recognizable embryo, not a tumor. In the case of the mature sperm the impossibility of even parthenogenetic development, much less tumor formation, is obvious.

*Hypothesis of Extragonadal Germ Cells.*—That the primordial germ cells of the developing embryo do not arise in the gonads has been accepted for many years. Where then do they come from? In 1870 Waldeyer suggested that they originate in a differentiated portion of the celomic epithelium that covers the urogenital folds. Subsequent work has indicated that this region is only the last way station of a much more extended journey. In 1914 Swift<sup>32</sup> demonstrated that the primordial germ cells of the chick embryo arise in a crescent-shaped region of germ wall entoderm at the anterior margin of the area pellucida. At first these cells are in the space between entoderm and ectoderm; with the appearance of the mesoderm they enter this layer and the developing blood vessels within it. Early in their course they migrate by ameboid movement, but subsequently they are carried in the blood stream to all parts of the embryo. Later the primordial germ cells become more numerous in the vessels of the splanchnic mesoderm. They continue to accumulate in the radix mesenterii and the celomic epithelium on both sides of the celomic angle; elsewhere they degenerate and disappear. Those in the celomic epithelium remain there until the formation of the gonad begins, when they gradually pass into that organ. This concept of a germ pathway or *Keimbahn* has been supported by numerous investigations, among them those of Reagan<sup>33</sup> and, more recently, of Dantschakoff.<sup>34</sup>

With this in mind it is interesting to consider the 11 cases of spontaneous teratoma of the chicken collected from the literature by Mashar<sup>35</sup> and supplemented by 2 additional cases of his own. In 5 cases the tumor originated within or on the surface of a kidney; in 3 a testicle was the primary site; in 1 case the tumor was found in an ovary, in 1 case in the mesentery and in 1 case in the abdominal air sac; in 2 cases the location was not given. These sites accord well with the regions of greatest accumulation of the primordial germ

32. Swift, C. H.: Am. J. Anat. **15**:483, 1914.

33. Reagan, F. P.: Anat. Rec. **11**:251, 1916-1917.

34. Dantschakoff, W.; Dantschakoff, W., Jr., and Bereskina, L.: Ztschr. f. Zellforsch. u. mikr. Anat. **14**:323, 1932.

35. Mashar, U.: Virchows Arch. f. path. Anat. **285**:155, 1932.

cells. The record of only a single case of ovarian teratoma is probably related to the fact that only 1 hen was included in the series.

To what extent primordial germ cells arrested in an extragonadal position may account for extragonadal teratoma of man is problematic. In the mouse embryo Everett<sup>36</sup> found primordial sex cells first in the gut entoderm, whence they migrated into the splanchnic mesenchyme and through the dorsal mesentery into the genital ridge. One might therefore expect the highest incidence of extragonadal teratoma to be within the intestinal wall and the mesentery. But this is not the case; however, a few cases of teratoma occurring in these regions have been reported. It is possible that teratoma of these and of retroperitoneal sites arise from "undescended" primordial cells, much as teratoma of the testis or the ovary originate from these cells after they have reached the gonads. Sacral and anterior mediastinal teratoma, however, are not accounted for by this hypothesis.

*Hypothesis of Fertilized Polar Bodies.*—Although still laboring under the mistaken belief in the fetal character of extragonadal teratoma, some investigators became dissatisfied with the "included twin" hypothesis. At the time when studies of the maturation of the egg cell were occupying the attention of embryologists, Marchand<sup>37</sup> and Bonriet<sup>38</sup> suggested that the polar bodies of the developing egg may be fertilized and pass into the medullary groove or other fissure, there to grow and differentiate into teratoma. After it was shown that polar bodies could not be stimulated to further development, this idea was dropped.

*Hypothesis of Displaced Blastomeres.*—With the discovery that the individual blastomeres up to and including the eight cell stage can each form a complete embryo, the scene was set for a new hypothesis. During the past fifty years the misplaced blastomere has been frequently called on to account for the presence of teratoma in various parts of the body.<sup>39</sup> The reasoning here is faulty because the early blastomeres can go on to independent development only if they are separated from the organizing influence of the adjacent cells. It has been shown experimentally that a translocation of embryonic cells while they are still totipotent, i. e., in the blastular stage, will result in their developing in conformity with the new environment. The problem may be approached from another angle. If two fertilized but yet uncleaved eggs of the salamander are united, they will form not conjoined twins but a single giant embryo. This demonstrates how great is the organiz-

36. Everett, N. B.: *J. Exper. Zool.* **92**:49, 1943.

37. Marchand, F.: *Mediastinal Geschwülste*, in Eulenburg, A.: *Real-Encyclopädie der gesamten Heilkunde*, Vienna, Urban & Schwarzenberg, 1897, vol. 15.

38. Bonnet, M.: *Ergebn. d. Anat. u. Entwicklungs gesch.* **9**:820, 1899.

39. Smith, L. W., and Stone, J. S.: *Ann. Surg.* **79**:687, 1924.

ing capacity of the early embryo—precisely the stage at which its failure has been postulated in the concept of a misplaced blastomere. Similar experiments on isolated blastomeres and fused eggs of rats have recently been carried out by Nicholas and Hall<sup>40</sup>; the results were identical with those reported for the salamander.

*The Morphogenetic Hypothesis.*—The attempt to explain the extragonadal appearance of teratoma on the basis of the misplaced totipotent cell has recently been revived by the application of the "morphogenetic theory" of Holmdahl.<sup>41</sup> This author amplified the work of Keibel, who first called attention to the fact that the tail and posterior segments of the trunk of the chick arise from an indifferent cell mass, the "tail-trunk bud" (*Rumpfschwanzknopse*). In addition to the posterior portion of the trunk, a narrow median region of the body also arises directly from the undifferentiated cell mass found in the dorsal blastopore lip (amphibian) or the anterior end of the primitive streak (chick and man). Holmdahl expressed the belief that the notochord, the underlying entoderm which forms the gut, and the basal portion of the neural groove differentiate directly from this tissue; they do not first pass through a germ layer stage. A number of other investigators, among them Vogt, Kingsbury<sup>42</sup> and Peter,<sup>43</sup> are in essential agreement with this hypothesis. The Belgian embryologist Pasteels<sup>44</sup> is one of its leading opponents.

Holmdahl has applied his hypothesis to the origin of teratoma,<sup>45</sup> placing special emphasis on the cases in which it arises in the sacral and the anterior mediastinal region. But dislocation of the totipotent cells should occur with approximately equal frequency throughout the mass of undifferentiated cells. His hypothesis therefore fails to account for the relatively high incidence of teratoma in the mediastinal and sacral regions. Moreover, since the indifferent cell mass is dorsal in position, the sacral location of teratoma can readily be explained but not the mediastinal, which is anterior to the heart. Nevertheless, further developments in this non-germ-layer hypothesis of the morphogenesis of the axial and caudal areas of the embryo will be followed with interest. When fully evaluated, it may form a useful part of the oldest and most widely applicable hypothesis of the genesis of extragonadal teratoma, namely, that it is due to a dislocation of tissues during early embryogenesis.

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40. Nicholas, J. S., and Hall, B. V.: *J. Exper. Zool.* **90**:441, 1942.
  41. Holmdahl, D. E.: *Anat. Anz. (supp.)* **88**:127, 1939. Holmdahl, D. E.: *Arch. f. Entwicklungsmechn. d. Organ* **139**:191, 1939.
  42. Kingsbury, B. F.: *J. Comp. Neurol.* **56**:431, 1932.
  43. Peter K.: *Ztschr. f. mikr.-anat. Forsch.* **36**:378, 1934.
  44. Pasteels, J.: *Arch. de biol., Paris* **48**:381, 1937.
  45. Holmdahl, D. E.: *Acta path. et microbiol. Scandinav.* **19**:603, 1942.

*The Hypothesis of Tissue Dislocation with Liberation of Organizers.*—Since the days of von Baer, embryologists have marveled at the complexity of the invaginations, delaminations and migrations of cell groups during embryogenesis. Small wonder that bits of tissue may be misplaced, particularly in regions where these processes are intricate! Because the most elaborate movements of cell groups occur only after the definitive germ layers have been formed, when these displaced tissues are no longer totipotent, many investigators were loath to seek in them the origin of such a complex malformation as teratoma. Readily, however, did they ascribe to tissue dislocations such less complex developmental faults as craniopharyngioma, sacral spina bifida and pilonidal cyst, branchial sinus, branchial fistula and mediastinal cyst.

As was detailed in the foregoing pages, experimental embryology has demonstrated the capacity of tissues to differentiate in directions other than that normally expressed during embryogenesis. Not only is a dislocated tissue subjected to the action of the organizers of its new environment, but its capacity to react to them may be altered. As a result, it may differentiate into structures that are wholly foreign to that region. In areas where dislocation of tissues may involve all three germ layers teratoma is often found. This concept may explain the genesis of extragonadal teratoma in most instances.

*Genesis of Sacral Teratoma.*—On the basis of his embryologic researches, Holtfreter<sup>46</sup> has associated human sacral teratoma with faulty gastrulation resulting in a mass of entomesoderm being attached near the caudal end of the entodermal tube. Experimentally Holtfreter<sup>47</sup> produced this abnormality by cultivating the blastulas of newts in a 0.35 per cent salt solution (Ringer's solution). In amphibians neither skin nor nerve tissue is found in these masses, which do not come in contact with ectoderm. However, in man, in whom gastrulation is considerably modified, contact of the entomesoderm with ectoderm occurs, and neural tissue is induced in the latter by the organizer activity of the underlying mesoderm.

#### GENESIS OF TERATOMA OF THE ANTERIOR MEDIASTINUM

Through the years, two hypotheses of the genesis of teratoma of the anterior mediastinum have had alternating popularity: (a) It is due to a disturbance of the development of the branchial clefts; (b) it is an included twin. During the greater part of the nineteenth century most cases of teratoma of the anterior mediastinum reported were examples of the cystic variety consisting almost solely of skin and its

46. Holtfreter, J.: Sitzungsb. d. Gesellsch. f. Morphol. u. Physiol. **42**:78, 1933.

47. Holtfreter, J.: Arch. f. Entwicklungsmechan. d. Organ **129**:669, 1933.

appendages. Pinders,<sup>48</sup> Pflanz,<sup>49</sup> Virchow<sup>50</sup> and Wilms<sup>51</sup> considered teratoma of this type to be derived from the branchial cleft. Collenberg,<sup>52</sup> because he found thyroid tissue in his case, implicated this organ. Marchand<sup>53</sup> found the thymus gland intimately associated with teratoma of the mediastinum in the case studied by him and therefore sought the origin of the growth in the thymus, suggesting a possible derivation from the corpuscles of Hassall.

In 1898 Ekehorn<sup>54</sup> reported 2 cases in which tissues of all three germ layers were readily demonstrated. He expressed the belief that such complex structure could not be explained on the basis of disturbed embryogenesis; instead he interpreted it as evidence of an included twin and similarly accounted for the complexity of specimens reported by previous authors. Among these was the first specimen of mediastinal teratoma to be recorded. This, reported by Gordon<sup>55</sup> in 1823, was remarkable for the possession of well developed teeth embedded in alveolar bone, which Ekehorn identified as a jaw. Askanazy<sup>56</sup> lent the weight of his authority to the theory of fetus in fetu, accounting for the presence of the included twin by assuming that a totipotent germ cell passed into the celomic cavity during early fetal life. Hattori<sup>57</sup> reported a case of teratoma wholly embedded in the thymus gland; he offered the intriguing suggestion that an ovum or a blastomere had passed into the third branchial pouch and from there wandered into the deeper portions of the mediastinum. In 1932 Harrington<sup>23</sup> reported a tumor of the anterior mediastinum which "represents the caudal portion of a parasitic fetus."

It has now become clear that the hair-containing cyst (dermoid) of the mediastinum is linked with the more complex teratoma by numerous intermediate forms and that all forms must be genetically similar. The fetal character of teratoma of the mediastinal and other sites has been disproved, and an origin within the branchial cleft appears more likely. The frequency of developmental faults in this region is indicated by the common occurrence of branchial fistula and sinus, often associated with small tabs of cartilage.

48. Pinders, W.: Ueber Dermoidcysten des vorderen Mediastinums, Inaug. Dissert., Bonn, J. Bach, Wwe., 1887; cited by Rusby.<sup>9</sup>

49. Pflanz, E.: Ztschr. f. Heilk. **17**:473, 1896.

50. Virchow, R.: Virchows Arch. f. path. Anat. **53**:444, 1871.

51. Wilms, M.: Deutsches Arch. f. klin. Med. **55**:289, 1895.

52. Collenberg, T.: Zur Entwicklung der Dermoidkystome, Inaug. Dissert., Breslau, F. W. Jungfer, 1869.

53. Marchand, F.: Ber. d. oberhess. Gesellsch. f. Nat. u. Heilk. **22**:325, 1833.

54. Ekehorn, G.: Arch. f. klin. Chir. **56**:107, 1898.

55. Gordon, J. A.: Med.-Chir. Tr. **13**:12, 1825.

56. Askanazy, M.: Verhandl. d. deutsch. path. Gesellsch. **11**:39, 1907.

57. Hattori, S.: Verhandl. d. jap. path. Gesellsch. **3**:130, 1913.

Teratoma of the mediastinum always occupies the region of the thymus gland and has been frequently found in intimate relation with this structure.<sup>58</sup> Schmincke<sup>59</sup> described a cherry-sized cyst in the lower left lobe of the thymus in a 31 year old woman; it was lined by "skin" and filled with hair and sebaceous material. In the 16 cases reported in the present series thymic tissue was found within the capsule in 4 instances; however, it must be emphasized that sometimes only portions of the teratoma were available for detailed microscopic study. An additional case, not included in the series, is that in which teratoma completely replaced the thymus of a 10 day old infant. No thymic tissue was found elsewhere. On histologic examination this growth was seen to contain well differentiated nerve tissues.<sup>60</sup>

Of all the branchiogenic structures, the thymus anlage is the most plausible site for the development of mediastinal teratoma. The thymus is the only branchiogenic organ that regularly descends into the anterior mediastinum. During the embryogenesis of the thymus there is intimate association of ectoderm and entoderm, accompanied by mesenchyme.

In man, most of the thymus is derived from the third entodermal pouch of the pharynx (fig. 22). The role of ectoderm in the formation of the thymus has been disputed. It is pertinent to the morphogenesis of mediastinal teratoma to consider this point. The third branchial cleft, an ectodermal derivative, opens onto the surface of the skin through the cervical sinus and becomes dilated at its distal end to form the cervical vesicle. Subsequently, the latter severs its cutaneous connection but remains in close contact with the third pharyngeal pouch. Thereafter, according to Weller<sup>61</sup> and other investigators, the cervical vesicle slowly degenerates and disappears, forming no part of the definitive thymus. Recently, however, Norris<sup>62</sup> reinvestigated the fate of the cervical vesicle in detail and found that it spreads over the surface of the entodermal thymus, forming an ectodermal cortical layer. Some of the ectodermal cells become disengaged and are displaced into the medulla by swarms of lymphocytes that are

58. Cordes: Virchows Arch. f. path. Anat. **16**:290, 1859. Rolleston, H. D.: J. Path. & Bact. **4**:228, 1897. Rénon, Delille, and Nandrot: Bull. et mém. Soc. anat. de Paris **82**:308, 1907. Schuster, H.: Beitr. z. path. Anat. u. z. allg. Path. **75**:403, 1926. Wolfsohn, G.: Arch. f. klin. Chir. **155**:680, 1929. Rose, E.: M. Clin. North America **14**:999, 1931. Struthers, R. R.: Canad. M. A. J. **26**:68, 1932. Marchand.<sup>53</sup> Hattori.<sup>57</sup>

59. Schmincke, A.: Pathologie des Thymus, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8, pp. 760-809.

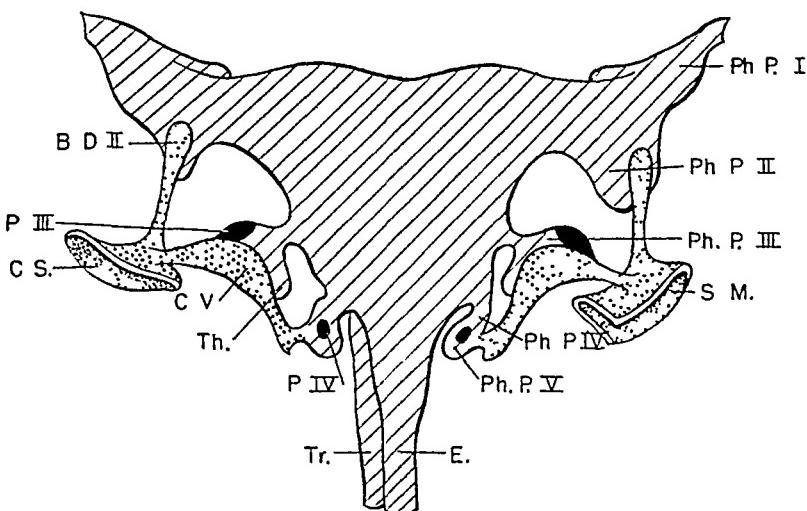
60. This case was made available to me by Major E. D. Peasley.

61. Weller, G. L., Jr.: Contrib. Embryol. **24**:93, 1933.

62. Norris, E. H.: Contrib. Embryol. **27**:191, 1938.

crowding into the cortex. The isolated ectodermal cells are at first recognizable by their large size and vesicular nuclei; subsequently, they form Hassall's corpuscles.

If Norris' conception of the origin of Hassall's corpuscles is correct, the thymus has the unique arrangement of ectodermal cells within a cell mass derived from entoderm. However, even if Hassall's corpuscles are entodermal in origin, it cannot be denied that the entodermal thymus at one stage in its development lies in intimate contact with the ectodermal cervical vesicle. The embryogenetic fault preceding the formation of teratoma may then consist of failure on the part of the cervical vesicle to disappear.



ECTODERM

ENTODERM

Fig. 22.—Diagram showing the pharyngeal pouches and their relation to the branchial clefts in a 6 week (12 mm.) human embryo, viewed from the dorsal aspect. *B. D. II* indicates branchial duct II; *C. S.*, the cervical sinus; *C. V.*, the cervical vesicle; *E*, the esophagus; *P. III* and *P. IV*, parathyroid glands III and IV; *Ph. P. I* to *V*, pharyngeal pouches I to V; *S. M.*, the orifice of the sinus; *Th.*, the entodermal anlage of the thymus; *Tr.*, the trachea. (After Hammar, J. A.: Zur groberen Morphologie und Morphogenie der Menschenthymus, Anat. Hefte 43: 201, 1911).

Partial degeneration of the cervical vesicle or of Hassall's corpuscles releases organizers (compare areas of necrosis as inductors of experimental testicular teratoma or boiled tissue as evocators) that act on the embryonic entodermal cells and the surrounding ectoderm. The capacity of even adult thymic reticulum cells to respond in this manner is shown by the occurrence of ciliated epithelium about the areas of necrosis which are frequently found in the dog's thymus.<sup>63</sup>

63. Bargmann, W.: Der Thymus, in Möllendorf, W.: Handbuch der mikroskopischen Anatomie des Menschen, Berlin. Julius Springer, 1943, pp. 1-172.

With reference to the presence of nerve tissue in teratoma of the thymus it may be noted that Politzer and Hann<sup>64</sup> observed in the early embryo an intimate relation between the cervical sinus and the ganglion nodosum of the vagus. This intimate connection was further emphasized by Kolmer,<sup>65</sup> who expressed the belief that some of the nerve cells in the ganglion nodosum may actually be derived from epithelium of the branchial cleft. Gilmour<sup>66</sup> has lately described an accessory left parathyroid gland III embedded in the ganglion of the trunk of the left vagus. No capsule separated it from the nerve tissue. Since parathyroid gland III arises from the third pharyngeal pouch together with the thymus (fig. 22), the report of Gilmour lends support to the belief that some of the nerve cells of the ganglion nodosum are derived from the cervical vesicle.

It seems probable that the site of origin determines the types of tissue which comprise teratoma. Thus, entodermal derivatives may be expected to be prominent in thymic teratoma. This is actually the case in the 10 specimens of benign teratoma of the present series. Bronchial and intestinal organoid structures are common, but of particular significance is the presence of well differentiated pancreas in 6 or possibly 7 specimens. On the other hand, in 30 specimens of ovarian teratoma examined by me, not once was pancreatic tissue observed. Similarly in 150 specimens of teratoma of the testis recently studied by another member of the staff, no pancreas was identified. Aberrant pancreas is not infrequently found throughout the gastrointestinal tract.<sup>67</sup> Lauche<sup>68</sup> stated that the entire foregut possesses the latent capacity to form pancreas. Therefore, the frequent presence of pancreas in specimens of teratoma of the anterior mediastinum may be ascribed to the important role played by part of the foregut, the third pharyngeal pouch, in their genesis.

One may conclude that teratoma of the anterior mediastinum has its origin in faulty embryogenesis of the thymus. Teratoma of this region may therefore be identified more precisely as teratoma of the thymus.

#### SUMMARY

The clinical course and morphologic characteristics of teratoma have been studied in 16 instances in which the growth occupied the anterior

64. Politzer, G., and Hann, F.: *Ztschr. f. Anat. u. Entwicklungs gesch.* **104**:670, 1935.

65. Kolmer, W.: *Ztschr. f. Anat. u. Entwicklungs gesch.* **87**:354, 1928.

66. Gilmour, J. R.: *J. Path. & Bact.* **52**:213, 1941.

67. Faust, D. B., and Mudgett, C. S.: *Ann. Int. Med.* **14**:717, 1940. Troll, M. M.: *Arch. Path.* **38**:375, 1944.

68. Lauche, A.: *Virchows Arch. f. path. Anat.* **252**:39, 1924.

mediastinum. In 10 of these cases the growth was benign and in 6 cancerous. All the patients fell within the military age group of 18 to 38 years; 15 were males; 1 was a female.

In the specimens of benign teratoma the most frequently encountered organoid structures were skin, "intestine," "bronchus" and "pancreas." The incidence of well developed pancreas in 6 of the 10 specimens is remarkable in view of its infrequent occurrence in specimens of teratoma of other regions.

The specimens of cancerous teratoma of this series were characterized by almost complete absence of ectodermal derivatives, such as skin or nerve tissue. The connective tissue was loose and cellular and may have undergone cancerous change. The cancerous epithelium was arranged as adenocarcinoma in each instance. Well differentiated organoid epithelial structures were absent. Metastases were found in 4 cases.

During the past half century the experimental analysis of morphogenesis has made important advances. Outstanding among these has been the development of the concept of the organizer which holds that substances ("organizers") liberated by one group of cells may determine the differentiation and the organization of other groups of cells.

The hypotheses of the genesis of teratoma have been examined in the light of advances in embryology. It is concluded that teratoma of the ovaries and the testes is due to abnormal growth and differentiation of undifferentiated precursors of the germ cells. Extragonadal teratoma, however, is the result of a local dislocation of tissues during embryogenesis.

Teratoma of the anterior mediastinum probably arises from tissue dislocations in the anlage of the thymus.

# Case Reports

## STRUMA OVARI

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THAT thyroid tissue occurs within the ovary has long been recognized. It occurs relatively frequently in association with dermoid cysts of that organ. Wynne, McCartney and McClendon,<sup>1</sup> in an analysis of 198 cases of ovarian teratoma and dermoid cysts reported in the literature, recorded thyroid tissue as present in 11.6 per cent. Koucky<sup>2</sup> reported an incidence of 19 per cent in 100 cases of ovarian dermoids. The total amount of thyroid tissue and the percentage relative to other tissue components vary within wide limits. The thyroid element may represent merely an incidental microscopic observation, or it may form a sizable portion of the ovarian mass, overshadowing the associated dermoid elements. The term "struma ovarii" has been applied when the thyroid component has formed a significantly large part of the tumor. Rarely, an ovarian tumor composed entirely of thyroid tissue is observed. Such a tumor might well be designated as pure struma ovarii. In an analysis of 50 cases of struma ovarii reported in the literature, Frankel and Lederer<sup>3</sup> noted 11 in which thyroid tissue formed the entire tumor. They reported 1 additional case of pure struma ovarii. Since then we have encountered reports of 15 other cases which we accept as instances of pure struma ovarii.<sup>4</sup> The data supplied in some reports are not sufficiently inclusive to establish the absence of other tissue components in the lesion. The publication of Dionisi and Ferraris<sup>5</sup> was not accessible to us. To the group of cases of pure struma we add case 1 in the following report:

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From the Department of Pathology, University of Pittsburgh, and the Presbyterian and Woman's Hospitals.

1. Wynne, H. M. N.; McCartney, J. S., and McClendon, J. F.: Am. J. Obst. & Gynec. **39**:263, 1940.

2. Koucky, J. D.: Ann. Surg. **81**:821, 1925.

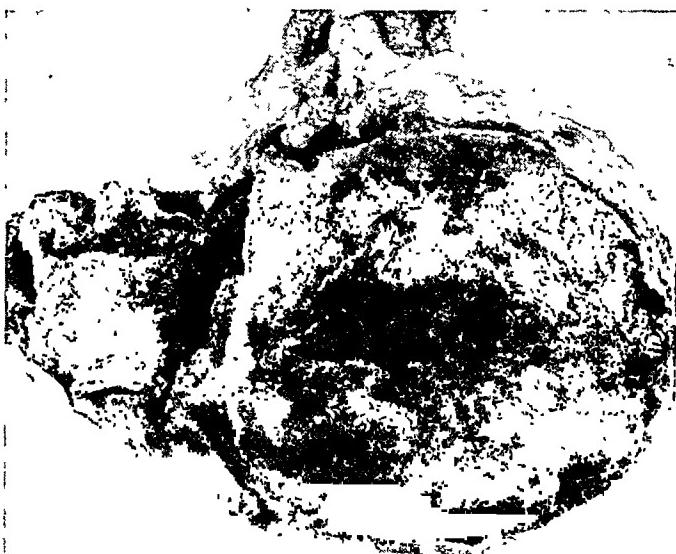
3. Frankel, J. M., and Lederer, M.: Am. J. Obst. & Gynec. **16**:367, 1928.

4. (a) Brown, A. L., and Shoor, M.: Am. J. Surg. **55**:173, 1942. (b) Wynne, McCartney and McClendon.<sup>1</sup> (c) Lyday, R. O.: Am. J. Surg. **25**:89, 1934. (d) Masson, J. C., and Mueller, S. C.: Surg., Gynec. & Obst. **56**:931, 1933. (e) Emge, L. A.: Am. J. Obst. & Gynec. **40**:738, 1940. (f) Shapiro, P. F.: Ann. Surg. **92**:1031, 1930. (g) Sailer, S.: Am. J. Clin. Path. **13**:271, 1943. (h) Cantor, P. J., and Kogut, B.: Am. J. Cancer **28**:760, 1936. (i) King, E. S. J., and Norris, J. H.: J. Coll. Surg. Australasia **3**:373, 1931.

5. Dionisi, H., and Ferraris, L. V.: Bol. y trab., Soc. de cir. de Córdoba **5**:179, 1944.

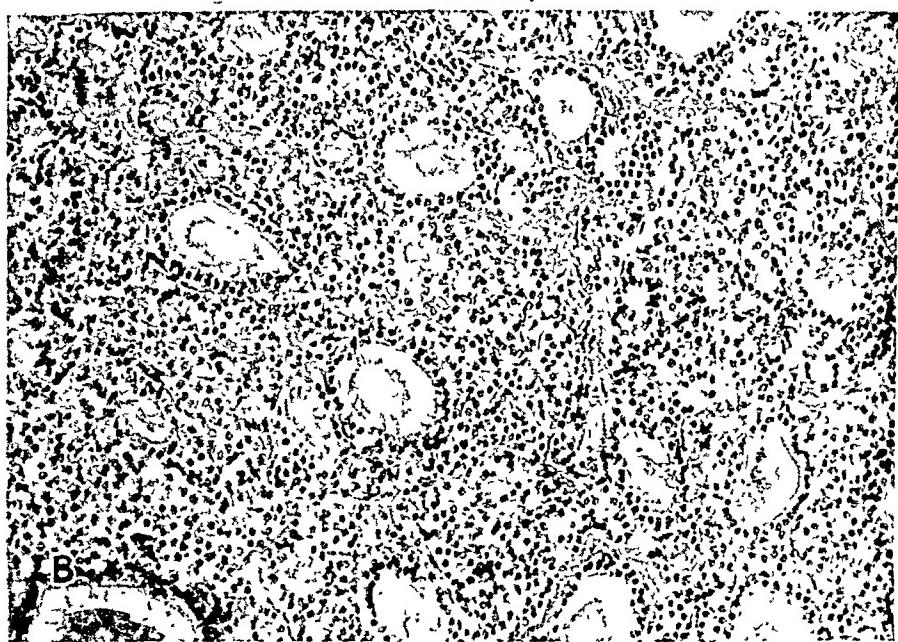
## REPORT OF CASES

CASE 1.—Mrs. H. M., a 37 year old white woman, was admitted to the Presbyterian Hospital, service of Dr. N. C. Ochsenshirt, Feb. 23, 1945. She complained of irregular and prolonged menstrual periods of two years' duration, associated with mild weakness and fatigue. She was admitted because of a mass



A

— 5 cm. —



B

Fig. 1 (case 1).—A, struma ovarii surrounded by an ovarian capsule. Atrophic ovarian parenchyma appears to the left. The fimbriated extremity of the fallopian tube is above. The photograph illustrates the glistening fleshy cut surface of the tumor. B, typical thyroid tissue composed of large and small follicles;  $\times 130$ .

in the pelvis, noted in the course of a premarital examination. There were no additional complaints and no evidence of hyperthyroidism.

Examination revealed nothing other than a firm, freely movable, nontender mass the size of an orange to the left of, and apparently attached to, the uterus, palpable only on bimanual pelvic examination. Laboratory examinations yielded normal results. The clinical impression was that of uterine fibroid, for which laparotomy was performed.

Approximately 2 liters of clear serous fluid was present in the peritoneal cavity. The mass noted on pelvic examination occupied the left ovary, which was removed along with a subserous adenomyoma 3 cm. in diameter attached to the anterior surface of the uterus. The peritoneum, the liver and other visible abdominal viscera were grossly normal.

After an uneventful convalescence, the patient was discharged on the twelfth postoperative day.

*Pathologic Examination.*—The ovary had been largely replaced by a solid ovoid tumor, 8 cm. in diameter, covered by an intact pale opaque ovarian capsule. On section, only atrophic remnants of ovarian parenchyma and an overdistended capsule enclosed the tumor (fig. 1A). The tumor tissue bulged slightly, was reddish brown, gelatinous, glistening and translucent. Numerous irregular opaque cellular-appearing areas were scattered throughout, their borders merging imperceptibly with the gelatinous matrix. Throughout the tumor were various-sized cysts containing colloid. Careful gross examination of closely spaced serial sections revealed no apparent variation in structure or appearance.

*Microscopic Examination.*—Several sections were made, and they all revealed thyroid tissue composed of follicles of fetal and adult type, the former predominating (fig. 1B). Solid columns, alveolar masses and small follicles containing little or no colloid bore striking resemblance to the so-called fetal adenoma of the thyroid gland. Scattered throughout were larger follicles with low columnar epithelium, appearing moderately hyperplastic. The enclosed colloid was acidophilic, and its edges were vacuolated and scalloped. Larger follicles contained more abundant colloid and were lined with cuboidal epithelium. The fibrous stroma was loose, edematous and in areas hemorrhagic. A loose edematous fibrous capsule separated the thyroid tissue from the surrounding ovarian cortex. Tissue other than thyroid was not demonstrable in the tumor.

To illustrate the much more common association of thyroid tissue with dermoid elements in struma ovarii we present a second case:

CASE 2.—Mrs. F. W., a 60 year old white woman, was admitted to the Woman's Hospital, service of Dr. E. M. Baker, Aug. 1, 1945, complaining of vaginal discharge and urinary incontinence, present intermittently since parturition twenty-five years previously. Examination revealed advanced inflammatory disease and prolapse of the cervix and an old laceration of the urethral sphincter. A large cyst occupied the position of the left ovary, which was freely movable and not tender.

General examination revealed nothing noteworthy. The thyroid gland was not enlarged. There were no signs or symptoms of hyperthyroidism. Laboratory and roentgen studies disclosed nothing of note. On August 6 a vaginal and urethral plastic operation was performed. One week later left salpingo-oophorectomy and appendectomy were done. The postoperative course was uneventful.

*Pathologic Examination.*—The ovary was enlarged and ovoid, measuring 10 cm. in its greatest diameter. It had a fluctuant consistency and was covered by a pale, opaque, smooth, intact capsule. The fallopian tube, attached to the ovarian

capsule, was grossly normal except for flattening and elongation, the result of pressure. Several small subcapsular cysts containing clear watery fluid caused slight nodulation of the ovarian mass. On section a large unilocular cystic cavity was exposed, distended with yellowish brown amorphous granular caseous material containing numerous hair shafts. The cyst and its contents formed the bulk of the mass. The cyst wall, for the most part, was thin and leathery, averaging 2 mm. in thickness. Its inner lining was wrinkled and opaque, resembling epidermis, and from its surface numerous hair shafts extended into the cavity of the cyst. No dermoid papilla was evident. At one pole there was a prominent thickening of the cyst wall, the result of the presence of a sharply circumscribed oval lobular solid mass measuring 6 cm. in its greatest diameter (fig. 2). Its cut surface was reddish brown and studded with minute colloid vesicles typically thyroid in appearance. Following evacuation of the cyst contents, the nodular mass of thyroid tissue formed an estimated 75 per cent of the total bulk of the specimen.

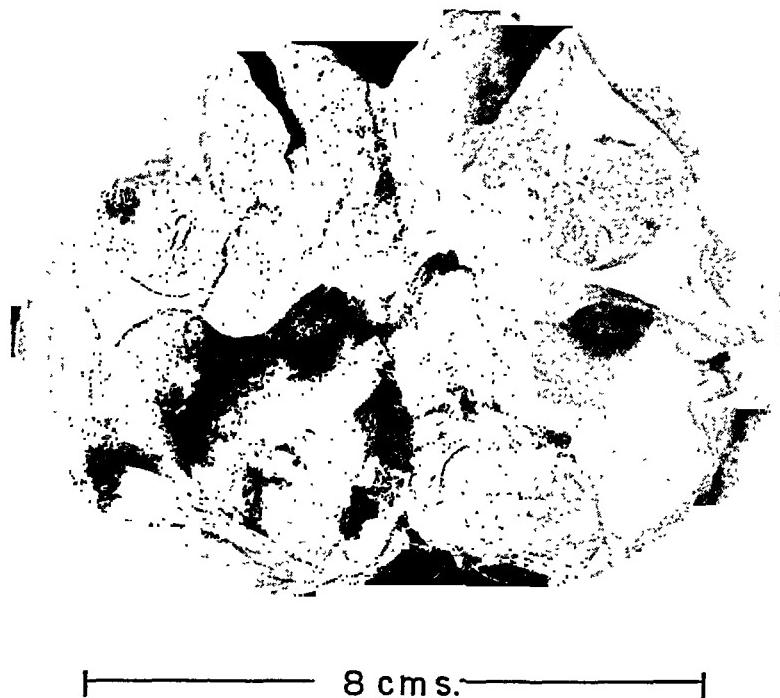


Fig. 2 (case 2).—Struma ovarii with dermoid cyst. The specimen has been hemisected and the cyst contents removed. The transected mass of thyroid tissue appears in the upper half of the object.

*Microscopic Examination.*—Sections revealed a thin, atrophic zone of ovarian cortex beneath a thin capsule. The bulk of the tissue was thyroid, composed of follicles which varied considerably in size. The majority were small, of the fetal type, and contained little or no colloid. The follicular epithelium was cuboidal in type. Other follicles were larger and contained normal amounts of old colloid. Still others formed small cysts, which were distended with colloid and lined by flattened epithelial cells. Infrequently the follicular epithelium assumed columnar characteristics, but the general picture was not that of hyperplasia. Areas of the stroma showed serous degeneration and small interstitial hemorrhages. A thin fibrous capsule separated the thyroid tissue from the surrounding ovarian parenchyma.

The cavity of the large central cyst was lined with stratified squamous epithelium, the outermost layers of which were cornifying and desquamating into the cavity of the cyst.

#### COMMENT

Although there was microscopic evidence of epithelial hyperplasia in case 1 and a large mass of ectopic thyroid tissue in both cases, the patients gave no clinical evidence of hyperthyroidism, and it must be assumed that the tissue was not physiologically overactive. Cases in which struma ovarii was associated with hyperthyroidism have been observed,<sup>6</sup> and reports of cancerous ovarian thyroid appear in the literature.<sup>7</sup>

Although several earlier investigators have questioned whether the tissue in such cases is truly thyroid, the overwhelming consensus of the present day accepts such an interpretation. The position was considerably strengthened by the work of Plaut,<sup>8</sup> who studied the pharmacologic and biologic activity of tissue from specimens of struma ovarii.

Thyroid tissue present in the ovary is generally regarded as a manifestation of teratomatous growth arising from totipotential germinal cells. The fact that other tissue elements occur in the ovary in the majority of cases lends support to such a concept. Not infrequently, one-sided overgrowth of the thyroid component renders the teratomatous nature inconspicuous, and unless the examination is most diligent and supported by numerous microscopic sections, other tissue components may be overlooked. It appears to be well established that pure struma, composed of thyroid tissue only, does occur, although rarely. In such cases the possibility of pressure atrophy, absorption and disappearance of other tissue elements must be conceded. The explanation of the selective overgrowth of thyroid tissue in a teratomatous ovarian neoplasm remains obscure.

#### SUMMARY

Two cases of struma ovarii have been observed, in one of which the tumor was composed entirely of thyroid tissue. It is probable that struma ovarii of the latter type is originally teratomatous and that the overgrowth of thyroid tissue ultimately destroys other tissue elements.

NOTE.—Following completion of this paper, several additional sections were made for teaching purposes from blocks of tissue in case 1. At one area within the ovarian thyroid there was a small acinus lined with columnar mucus-secreting epithelium, distinctly not thyroid in type. Although this nullifies the "pure" aspect of the struma, it supports the concept that struma ovarii is originally teratomatous.

6. (a) Moench, G. L.: *Surg., Gynec. & Obst.* **49**:150, 1929. (b) Emge.<sup>4e</sup>  
(c) Gusberg, S. B., and Danforth, D. N.: *Am. J. Obst. & Gynec.* **48**:537, 1944.

7. Wynne and others.<sup>1</sup> Emge.<sup>4e</sup> Shapiro.<sup>4f</sup>

8. Plaut, A.: *Am. J. Obst. & Gynec.* **25**:351, 1933.

## HEPATIC ABSCESS COMPLICATING ATRESIA OF THE SMALL INTESTINE OF A NEWBORN INFANT

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HEPATIC abscess is comparatively rare in children and to encounter a well established abscess in a newborn infant is uncommon indeed. No instance of abscess of the liver of a newborn infant was found reported, but a report of abscess of the liver occurring at 6 weeks was found.<sup>1</sup>

The following report concerns a newborn infant with a hepatic abscess present at birth, who lived for thirty-nine hours.

### REPORT OF A CASE

The mother, a patient of Dr. Edes Alsup, is Japanese, "aged 32, gravid five times, with four normal living female children." Although she had had nausea in early pregnancy, with vomiting on a few occasions, the course of pregnancy had been normal except for swelling of the ankles and "spilling of small amounts of water" for one week prior to admission.

On admission the mother showed slight edema of the ankles; the blood pressure was 140 systolic and 90 diastolic; she was "fully dilated" and in labor. The membranes were ruptured artificially, and the second stage was completed in about twenty-one minutes after admission. No gross abnormalities were observed in the placenta. She was discharged on the fifth day. Her blood showed: red cells, 4,970,000; hemoglobin content, 13.8 Gm.; white cells, 6,800, with 39 per cent polymorphonuclear leukocytes, 58 per cent lymphocytes and 3 per cent eosinophils. The feces revealed no parasites.

The baby was a girl, born in fair condition, who cried spontaneously. The whole body was described as hard and nonpitting, with an edematous appearance. The color became cyanotic, respiration was somewhat difficult, but oxygen improved the color. However, respiration remained grunty. After four hours, the swelling of the face and the hands was relieved somewhat, but the abdomen and the lower extremities remained edematous. Later, the edema improved further, urine was discharged in small amounts, vomiting of small amounts of bright green fluid followed and terminally there was a macular rash over the entire body, especially over the lower extremities and the abdomen. The cyanosis increased, the patient failed to respond to oxygen therapy and artificial respiration, and death occurred about thirty-nine hours after birth.

The infant's blood showed: erythrocytes, 5,710,000 per cubic millimeter; hemoglobin content, 16.5 Gm.; leukocytes, 20,500 per cubic millimeter, with 44 normoblasts per hundred cells in the differential count. A corrected estimate of the leukocyte count would be approximately 12,000 per cubic millimeter. The differential count showed polymorphonuclears 51 per cent, lymphocytes 38 per cent, eosinophils 4 per cent, monocytes 3 per cent and myelocytes 4 per cent. The erythrocyte count showed many microcytes and a few macrocytes with some

From the Department of Laboratories, Queens Hospital.

1. Kutsunai, T.: Am. J. Dis. Child. 51:1385, 1936.

polychromasia. The infant's blood type was A and was Rh positive. (The mother's blood was of type AB and Rh positive.)

The blood nonprotein nitrogen of the baby was 31 mg. per hundred cubic centimeters; the blood sodium chloride, 499 mg., with 260 mg. as chlorine. The van den Bergh reaction showed 3 units, and the Weltmann coagulation band, 6.

Roentgen examination showed three large masses in the abdomen: One lay under the lower seven ribs on the right side; another extended well down into the flank, apparently under the one just mentioned and down into the pelvis and laterally beyond the midline to the left; a third mass occupied the entire lumbar area, reaching over to the left for a distance of about 1 cm. beyond the vertebral line and apparently lying outside the pelvis. The left side of the abdomen was obscured and filled with gaseous shadows. No interpretation was given.

*Autopsy* (eight hours after death).—The brain was edematous. The lungs sank quickly when placed in water and showed marked atelectasis. The heart, the thymus, the esophagus and the thyroid disclosed no marked changes. The abdomen presented generalized plastic peritonitis with considerable matting of the intestines because of fine adhesions. The liver was densely adherent to the diaphragm, and the upper mass observed in the roentgenogram lay entirely within the right lobe of the liver. This mass was an abscess filled with dark brownish, cloudy fluid with a slight greenish tinge. The "capsule" of the abscess was thick and well developed, varying from 1 to 2 mm. in thickness. After evacuation the interior was relatively smooth except for a small amount of brownish granular deposit. The fluid was of a foul odor like that produced by the action of the colon bacillus. The abscess involved two thirds of the right lobe of the liver. The gallbladder was small and contracted and occupied its usual bed. The bile duct was normal in its relations. Culture of the fluid from the hepatic abscess showed many coliform organisms. No amebas were found. Microscopically, the wall was dense and fibrotic with calcific deposits and degeneration products of long standing.

The middle mass in the roentgenogram was a peritonealized abscess containing approximately 2 ounces (60 cc.) of fluid similar to that in the hepatic abscess. It was densely adherent to the surrounding structures and was separated with some difficulty. So far as could be determined, it did not communicate with any of the viscera. This mass occupied the greater portion of the right side of the abdomen, lying beneath and attached to the liver, and descending into the pelvis.

The third mass was a bulbous dilatation of a blind pouch above the midbowel area, which contained a foul-smelling, thin, brownish fluid of colon bacillus odor. Stretched over this mass was a threadlike atretic portion of the intestine, which extended 3 cm. beyond the margins of the mass. Above the bulbous dilatation was another elongated sacculation on one side. Beyond the atretic portion the ileum was collapsed and empty except for a large Meckel's diverticulum (2 by 0.75 cm.) which was found about 4 cm. below the obliterated area. The jejunum and the duodenum, on the other hand, except for the bulbous dilatation, were of average diameter and contained a moderate amount of liquid of yellowish brown (bile-stained) color and about ten soft pellet-like masses of greenish mucus. The ligament of Treitz acted as an obstructive band, with the result that there was a complete twisting of the intestine on itself with a loop lying underneath it.

The colon was collapsed throughout and was about one third the diameter of the small intestine. There was no evidence of meconium in the colon. The stomach was normal. The generalized peritonitis and adhesions have been mentioned.



A



B

A, roentgenogram showing an intrahepatic abscess, a subhepatic abscess and a bulbous dilatation of the small intestine above the atretic portion. B, upper surface of the liver showing a collapsed abscess.

The spleen, the kidneys, the adrenal glands, the fallopian tubes and the ovaries presented no gross abnormalities.

The diagnoses were: generalized peritonitis with adhesions; large hepatic abscess of the right lobe of long standing; right subhepatic peritonealized abscess; atresia of the jejunum with bulbous dilatation; Meckel's diverticulum; pulmonary atelectasis.

The gaseous shadows shown in the roentgenogram apparently did not arise from intestinal ballooning but from peritoneal pockets of gas produced by bacterial action. Figure *B* shows in the liver of the newborn infant a well defined abscess possessed of a definite "fibrous capsule" containing calcific deposits. The atresia of the jejunum had not led to as much general dilatation as might have been anticipated from a blind pouch deformity, but this was probably because the baby had not been fed anything beyond a small amount of water. The atresia probably played a prominent role in the production of intestinal obstruction in prenatal life, with probably a focus of healed perforation, followed by a hepatic and a peritonealized abscess. That the bulbous dilatation represented another dilated diverticulum is a possibility. There had apparently been reactivation of the peritonitis as a terminal event.

#### SUMMARY

A newborn infant with atresia of the small intestine had, in addition, a subhepatic abscess and a large abscess of the liver with calcific deposits in the fibrous wall.

## Notes and News

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**Appointments.**—Colonel Ashley W. Oughterson, of the Army Institute of Pathology, Washington, D. C., recently medical aide to General Douglas MacArthur and formerly clinical professor of surgery at Yale University, is now executive vice president of the American Cancer Society.

F. R. Dieuaide, formerly clinical professor of medicine at Harvard Medical School, has assumed the directorship of the Life Insurance Medical Research Fund. The purpose of this fund is to make grants to universities and medical schools for research on diseases of the heart and related conditions.

D. Murray Angevine, formerly a member of the department of pathology in Cornell University, has assumed the professorship of pathology in the University of Wisconsin, succeeding Charles H. Bunting, retired.

Brigadier General R. A. Kelser, of the United States Army, is retiring from active duty to become dean and professor of bacteriology of the College of Veterinary Medicine of the University of Pennsylvania. General Kelser has served for nearly eight years as director of the veterinary division of the Surgeon General's Office.

Eugene Hildebrand Jr., formerly of the department of pathology of Passavant Hospital, Chicago, is now pathologist and director of laboratories of the Great Falls Clinic, Great Falls, Mont.

S. E. Gould, pathologist to the Wayne County general hospital, Eloise, Mich., is now the editor of the *American Journal of Clinical Pathology*, succeeding I. Davidsohn, resigned.

Edith Sproul, New York, recently has been appointed to a professorship in pathology at the American University, Beirut, Lebanon.

**Society News.**—The American College of Surgeons will hold its thirty-second clinical congress at the Waldorf-Astoria Hotel, New York, Sept. 9 to 13, 1946.

The American Association for the Study of Goiter will hold its annual meeting at the Drake Hotel, Chicago, June 20 to 22.

At the meeting of the American Association of Pathologists and Bacteriologists in Chicago, March 8 and 9, W. D. Forbus was elected president, M. H. Soule vice president, H. T. Karsner secretary and A. R. Moritz treasurer. At its next annual meeting the association will be the guest of the Jefferson Medical College, Philadelphia, on the Friday and Saturday preceding the meetings of the Federation of American Societies for Experimental Biology. At this meeting a symposium will be conducted on "Necrotizing Hepatic Injury and Sequels," with Lieutenant Colonel Balduin Lucké as referee.

**Fellowships in Public Health.**—Physicians interested in training for full time public health positions should write to the State Department of Health, Albany 1, N. Y.

**Hall of Fame.**—The late Major Walter Reed (1851-1902), the chairman of the United States Army Commission which in 1900 by experiments demonstrated that yellow fever is caused by a filtrable virus and is transmissible to man by the mosquito *Aedes aegypti*, has been elected to the Hall of Fame of New York University.

**Deaths.**—Harold Eugene Robertson, professor of pathology at the University of Minnesota Graduate School and formerly head of the section on pathologic anatomy at the Mayo Clinic, died on March 8, aged 67.

**Life Insurance Medical Research Fund.**—One hundred and forty-six life insurance companies in the United States and Canada have cooperated to establish this fund in support of fundamental research bearing on cardiovascular disease, including rheumatic fever, hypertension, arteriosclerosis and allied disorders. To assist the directors of the fund in making grants, an advisory council has been appointed with the following membership: Francis G. Blake, chairman; Ernest W. Goodpasture, A. Baird Hastings, Eugene M. Landis, Robert F. Loeb, C. N. H. Long, Seeley G. Mudd and Cecil J. Watson. Grants will be made for periods according with the specific requirements of the research problems. Applications for grants may now be made and should be transmitted in duplicate through the administrative officer of the institution making application. Requests for grants should include a description of the proposed research, a budget, and the date when funds are desired. Applications received by Feb. 1, 1946 will be given consideration at a meeting of the council to be held on or about March 1, 1946. Address Francis G. Blake, Chairman, Advisory Council, 333 Cedar Street, New Haven 11, Conn.

## Books Received

**Kettle's Pathology of Tumors.** By W. G. Barnard, F.R.C.P., professor of Pathology of the University of London and at St. Thomas's Hospital Medical School and director of pathology at St. Thomas's Hospital, London, and A. H. T. Robb-Smith, M.A. Oxon, M.D. London, Nuffield reader in pathology at the University of Oxford and director of pathology at Radcliffe Infirmary. Third edition. Pp. 318, with 191 illustrations. Price, \$5.50. New York: Paul B. Hoeber, Inc., 1946.

First comes a section on the biology of tumors. Tumor is defined as an autonomous new growth which is "unlimited, progressive, purposeless, and uncontrolled." This description does not apply fully to all forms of benign, or noncancerous, neoplasms. The authors discuss clearly and instructively the general structure and growth of tumors, the differences between noncancerous and cancerous tumors ("innocency and malignancy"), the characteristics of cancer, its dissemination, its experimental induction and causes and the cancer theories. The general pathology of tumors is the subject of the second section. Comments on the nomenclature and on the histologic and embryologic classifications are followed by descriptions of the microscopic structure of tumors grouped as follows: innocent connective tissue tumors; sarcoma; innocent epithelial tumors (papilloma and adenoma); carcinoma; tumors of the nervous system; melanoma; endothelioma; teratoma. The third part deals summarily with the special features of tumors as they occur in various tissues and organs. The few lines about carcinoma of the larynx (pages 226 and 227) do not reflect the progress in its treatment since 1936, which is the date of the article to which readers are referred for an analysis of the results of its treatment. The point of origin in most cases is a true vocal cord and not the piriform sinus. On page 233 one reads: "Ahlbom has suggested that pharyngeal carcinoma in women only occurs in those suffering from chronic hypochromic anaemia with atrophy of the mucous membrane." "Only" should be "mostly" or some word of like meaning. Most of the illustrations are microscopic drawings by Kettle himself "to show clearly the points described," but at this time and place they look strange—well made, it is true, but unreal and idealized. There are many unfamiliar words, especially in the discussions of classifications. The writing would have been simpler if the word cancer had been used in place of "malignant tumor," "malignancy," "malignant neoplastic disease." "Cancer" is now used to designate cancerous neoplasms so generally in common parlance, in names and in popular and nonmedical, as well as scientific, literature that there should be no hesitancy any longer to use it in textbooks wherever its use is indicated. On the whole, the book continues to be an excellent introduction to the study of tumors, better suited perhaps, on account of its terminology, for British than for American medical students.

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## CHANGES IN THE CENTRAL INCISORS OF HYPOPHYSECTOMIZED FEMALE RATS AFTER DIFFERENT POSTOPERATIVE PERIODS

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AND

DANIEL A. COLLINS, D.D.S.

SAN FRANCISCO

AND

MIRIAM E. SIMPSON, M.D., and HERBERT M. EVANS, M.D.  
BERKELEY, CALIF.

EXTENSIVE studies on the relation of the pituitary gland to skeletal growth have led naturally to investigations of the relation to the development of the teeth. In the rat, hypophysectomy after 1 month of age results in immediate cessation of skeletal growth, including regressive changes in the cartilaginous epiphyseal plate, disappearance of bone trabeculae, increase of the fat content in the marrow of the epiphysis and the diaphysis and "sealing off" of the epiphyseal cartilage by bone. For a recent and complete account of the changes in the osseous system of the rat at different intervals after hypophysectomy, reference may be made to Becks, Simpson and Evans.<sup>1</sup> A brief review of earlier work on the subject is also contained in that paper.

The changes in the incisor of the white rat following hypophysectomy were first observed histologically and roentgenographically by Schour and Van Dyke.<sup>2</sup> These authors made postmortem studies of 23 completely hypophysectomized rats which had been 36 to 64 days old at the time of operation and which were dead 2 to 15 months after operation. These animals were classified into three groups according to the roentgenographic and histologic observations:

Group I comprised 8 animals which lived from 2 to 6 months after the operation and which presented characteristic early changes: The

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This study was conducted under grants made by the American Foundation for Dental Science, the California State Dental Association, the Research Board of the University of California, the W. K. Kellogg Foundation, Meharry Medical College, and the Rockefeller Foundation in New York.

1. Becks, H.; Simpson, M. E., and Evans, H. M: Anat. Rec. 92:109, 1945.
2. Schour, I., and Van Dyke, H. B.: Am. J. Anat. 50:397, 1932.

contour of the labial surface of the incisor was composed of two segments ". . . an anterior larger segment of a small circle and a posterior smaller and flatter segment of a larger circle." A sharp indentation of enamel in the region of the junction of the apical and the middle third, and enamel globules projecting on the surface, were seen in the incisors of this group. Folding of enamel epithelium was a feature, and hypoplasia of enamel occurred sometimes to the extent that in spots no enamel was formed; at these places the enamel epithelium met the dentin surface. This folding was also observed in the roentgenograms.

Group II consisted of 8 animals which lived 6 to 10 months after the operation and showed intermediate changes in the apical portion of the incisor: Irregular contour of the labial surface at the apical end and partial absence of enamel formation were observed. The enamel matrix showed deep slits with epithelial and connective tissue ingrowth and partial degeneration of the enamel epithelium. The dentin had many tubular spaces, with complete perforation in some places. The enamel and the dentin on the labial surface were folded in the most posterior portion of the incisor.

Group III comprised 7 animals that lived from 10 to 15 months after the operation and presented advanced changes: Here calcification of the enamel in the apical region was incomplete and its labial outline irregular. The ingrowths were more pronounced, and multiple foldings (with three or four deep folds and a few minor folds) had formed, accompanied by severe displacement of Hertwig's sheath. The enamel epithelium was severely degenerated, and with the exception of a narrow slit the pulp was almost entirely obliterated. This condition was described as a result of continued dentin formation and lack of tooth eruption.

These authors summarized the findings of Groups II and III as follows: "The radiograph of the incisor of the hypophysectomized rat that lived a given period after the operation gives a constant pathognomonic picture". It must be noted that the conclusions of Schour and Van Dyke for groups II and III were based on 15 rats. These included 10 rats which died during the course of their study.

Our purpose in the present paper is to report a restudy of the question of the effect of hypophysectomy on tooth structures. This study was made with a larger number of animals, representing a greater range of time after the operation.

#### EXPERIMENTAL MATERIAL

One hundred and forty-one female rats<sup>3</sup> of the Long-Evans strain were hypophysectomized<sup>4</sup> when they were between 26 and 28 days of age. Only animals

3. A preliminary report on part of these animals is contained in the descriptions of hypophysectomized controls to experiments on the effects of hormone

in which the operation was complete have been included in this report. The completeness of operation was judged by the following criteria:

1. Growth curve: Little if any gain is noted in body weight and length after operation.
2. Muscular tone: After long postoperative periods the muscle tone is poor.
3. Condition of hair: The hair is soft, fluffy and long. The coarse guard hairs observed in normal animals are lacking.
4. Estrous cycles: There is complete suppression, and the vagina is closed.
5. At autopsy: The thyroid gland, the cortex of the adrenal gland and the gonads are atrophic.
6. The sella turcica (examined with dissecting binoculars) shows no remains of the gland.
7. Serial sections of any suspicious fragments of tissue in the sella turcica reveal no hypophysial remnants.

The rats were killed from 6 to 664 days after operation (table 1) and the skulls roentgenographed. The skulls of half of the older animals, those with a postoperative period between 316 and 664 days, were prepared for histologic studies. The material was fixed in neutral solution of formaldehyde U. S. P. (1:10), decalcified in 5 per cent nitric acid, embedded in pyroxylin, and the sections stained with hematoxylin-eosin.

#### RESULTS

*Roentgenographic Aspect.*—The study of roentgenograms of the skull disclosed changes in the incisors which increased in severity with increase in length of the postoperative periods. During the shorter periods, 6 to 12 days after operation, no abnormal changes could be detected (fig. 1A). After 14 days, thickening of the dentin on the labial side of the upper incisor (fig. 1B) was observed; this represents the earliest effect of hypophysectomy on the incisors. It was present in all animals 14 or more days after the operation and is designated as stage 1. At 87 days<sup>5</sup> after hypophysectomy thickening of the lingual dentin became definite (fig. 1C); it was present in all animals after this postoperative interval and constitutes stage 2. After 100 days an abrupt increase in the curvature of the apical region on the labial side was seen; this will hereafter be described as a distortion of the apical curvature (fig. 1D). This change characterizes stage 3. Definite folding of the apical end of the upper incisor was seen for the first time 144 days after

therapy on the teeth of hypophysectomized rats (Collins, D. A.: The Effect of Experimental and Endocrine Disturbances on Dental Structures of Dogs and Rats, Thesis in partial fulfilment of the requirements for the Degree of Master of Science in Dentistry, University of California, 1944).

4. The method of operation was a modified Smith parapharyngeal approach (Smith, P. E.: J. A. M. A. 88:158, 1927; Am. J. Anat. 45:205, 1930).

5. Because of the rather long interval of time between the postoperative periods of forty-three and eighty-seven days for which no observations are available, it must be considered possible that a lingual thickening may occur before eighty-seven days.

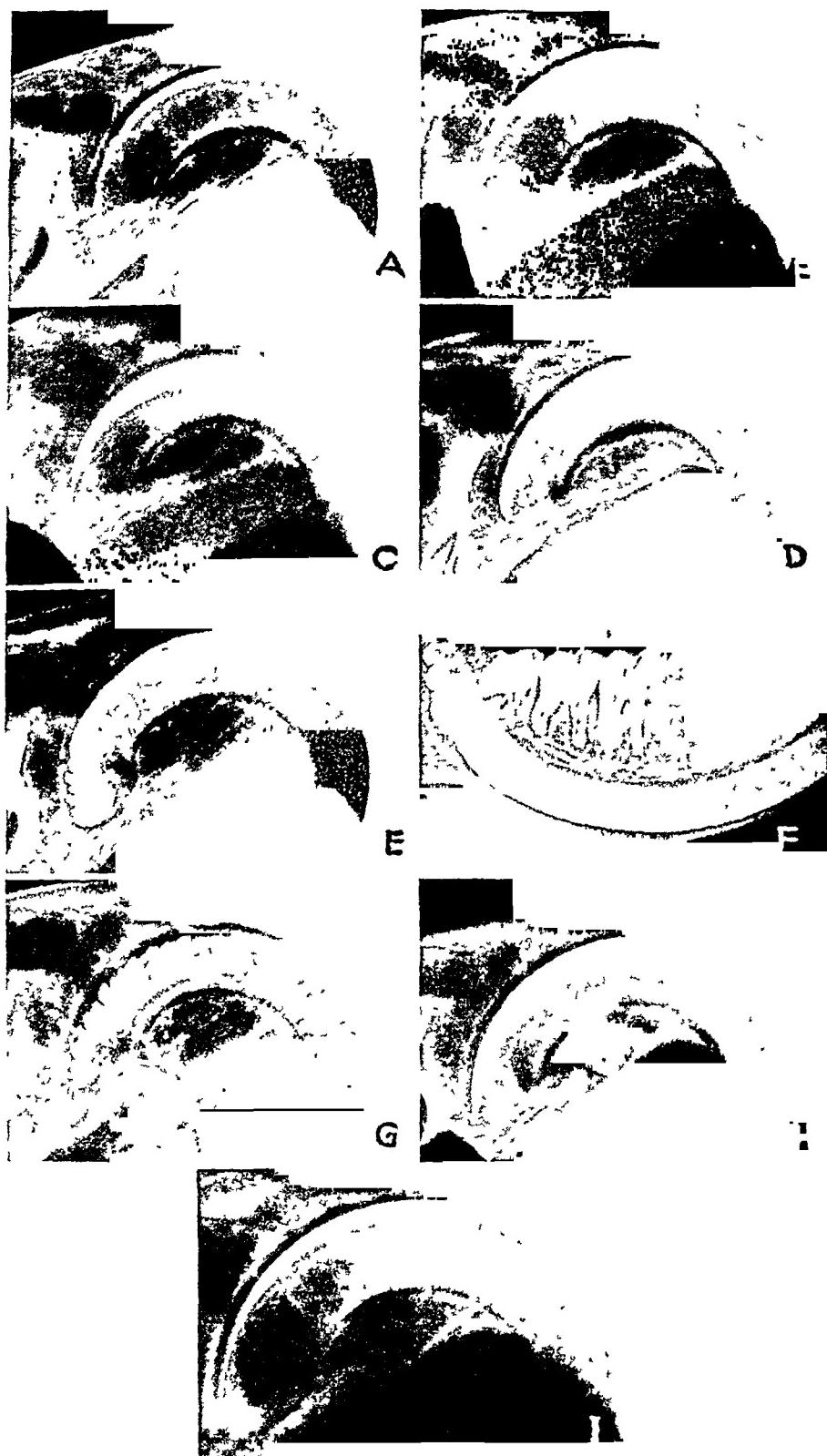


Figure 1

*(See legend on opposite page)*

the operation (fig. 1E) and is called stage 4. Folding of the apical end of the lower incisor (fig. 1F) was not observed before 205 days and even then occurred only occasionally; this change characterizes stage 5. Folding was never observed in the lower incisor only. Thus the roentgenographic changes fall into five definite stages. While the first stage occurred in all animals 14 days or more after operation and the second in all animals 87 days or more after operation, the frequency of stages 3, 4 and 5 varied considerably, indicating that these changes are by no means constant. A detailed analysis of the changes as seen in roentgenograms is given in table 1.

Deviations from these five distinct stages are represented in figure 1G and H. G shows that folding is not always restricted to the apical end but extends sometimes to the central portion of the incisor. H indicates that the incisors of some rats killed after the same interval (497 days) as those depicted in figure 1E, F and G showed only the changes described for stages 1 and 2. Figure 1I, showing the upper incisor of a normal control rat 449 days of age at autopsy, is included for purposes of comparison.

Roentgenograms of whole skulls (shown in fig. 2) allow comparison of the changes occurring after long postoperative periods. Figure 2A is a reproduction of the roentgenogram of the skull of a 458 day old normal control rat. The skull of a hypophysectomized rat killed 376 days after the operation (fig. 2B) is seen to be distinctly smaller, with the incisors shorter and smaller, than those of the normal control. However, it is interesting to observe that this animal, which presented changes typical of approximately one third of the 27 rats of its group (table 1), did not show any folding of the apical end of upper or lower incisors. The thickening of the labial and the lingual dentin nevertheless was distinct. In another animal, killed 349 days after the operation (fig. 2C), a severe apical folding had occurred in the upper incisor but not in the lower. Finally, figure 2D shows the skull of an animal 574 days after operation in which the apical foldings and the obliteration

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#### EXPLANATION OF FIGURE 1

Roentgenograms of incisors of hypophysectomized female rats taken at different postoperative periods (operation at the twenty-sixth day of age) ( $3\frac{1}{2}$  times natural size): A, postoperative period 12 days; age at autopsy 38 days. Note absence of structural changes. B, postoperative period 14 days; age at autopsy 40 days. Stage 1. C, postoperative period 87 days; age at autopsy 113 days. Stage 2. D, postoperative period 100 days; age at autopsy 126 days. Stage 3. E, postoperative period 497 days; age at autopsy 523 days. Stage 4. F, postoperative period 497 days; age at autopsy 523 days. Stage 5. G, postoperative period 497 days; age at autopsy 523 days. Note that the folding extends over the apical half of the incisor. H, postoperative period 497 days; age at autopsy 523 days. Note absence of folding. In spite of the long postoperative period, only stages 1 to 3 can be seen. I, incisor of a normal control rat whose age at autopsy was 449 days. Compare it in size with the incisors of the hypophysectomized rats.

TABLE I.—Roentgenographic Analysis of Changes of the Incisors of Hypophysectomized Female Rats Following Different Postoperative Periods (Operation at Twenty-Sixth to Twenty-Eighth Day of Age)

Rats	Post-operative Days	Age at Autopsy, Days	Stage 1:						Stage 2:						Stage 3:						Stage 4:					
			Thickening of Dentin			Distortion of Apical Curvature Without Folding			Distortion of Apical Curvature With Folding of Upper and Lower Incisor			Distortion of Apical Curvature With Folding of Upper Incisor			Distortion of Apical Curvature With Folding of Upper and Lower Incisor			Distortion of Apical Curvature With Folding of Upper and Lower Incisor			Groups Observed by Sehouer and Van Dyke					
			Labial		Lingual	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%	Rats	%			
4	6	32-34	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
4	8	31-36	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
4	10	36-38	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
4	12	38-40	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
3	11	49	3	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
3	16	44	3	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
3	18	72-76	5	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
5*	30	66-68	5	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
5	37	63-64	5	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
6	43	69	5	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
5	87	114	6	100	4	80	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
1	91	119	1	100	1	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
7	100	126-127	7	100	7	100	1	14	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
1	114	170-172	4	100	4	100	2	50	1	25	..	..	..	..	..	..	..	..	..	..	..	..	..			
22	205-210	231-237	22	100	22	100	6	27	6	27	1	27	1	27	1	27	1	27	1	27	1	27	1			
4	289-302	315-339	4	100	4	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
27	316-376	342-404	27	100	27	100	3	11	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14			
1	442	468	1	100	1	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
0	497	523-535	6	100	6	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
8	505-519	531-547	8	100	8	100	2	25	4	65	4	65	4	65	4	65	4	65	4	65	4	65	4			
10	534-544	560-602	10	100	10	100	1	10	1	10	5	10	5	10	5	10	5	10	5	10	5	10	5			
2	617	645	2	100	2	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
2	615-634	671-692	2	100	2	100	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..			
141	Occurrence of stage after first seen	125	125	100	98	99	99	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14			

\* This group was hypophysectomized at 57 days of age.

tions of the pulp tissues of upper and lower incisors were extreme. Even though the dental changes vary considerably in figure 2B, C and D the skulls are seen to be approximately the same size.

*Histologic Aspects.*—Since roentgenograms of hard structures frequently do not disclose minute pathologic changes, the skull of the 26 animals with the longest postoperative periods were prepared for histologic examination. They were selected without consideration of roentgenographic findings. All had lived from 316 to 664 days after the operation and were divided into two groups (table 2); Group I

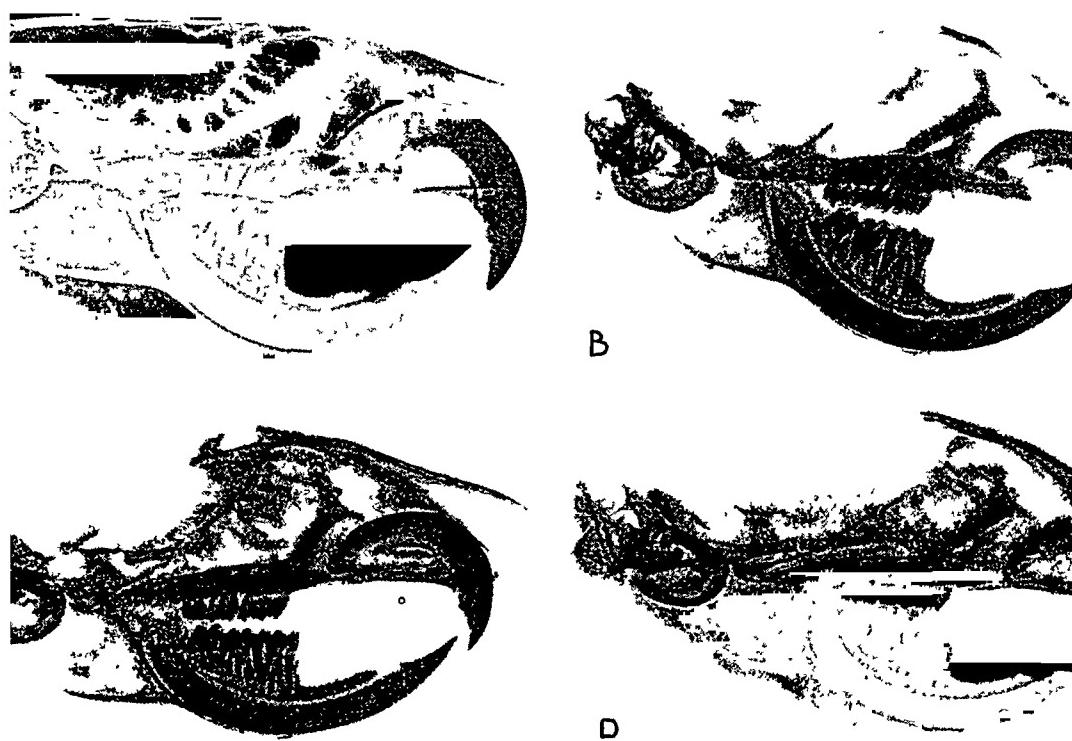


Fig. 2.—Roentgenograms of the right halves of the skulls of normal and hypophysectomized rats (2 times the natural size): A, normal control; age at autopsy 458 days. B, hypophysectomized rat; postoperative period 376 days; age at autopsy 403 days. Note absence of apical foldings in the upper as well as in the lower incisor. C, hypophysectomized rat; postoperative period 349 days; age at autopsy 372 days. Apical foldings occur only in the upper incisor. D, hypophysectomized rat; postoperative period 574 days; age at autopsy 603 days. Folding occurs in the lower as well as in the upper incisor.

included 11 animals killed 316 to 442 days after operation and corresponded to the oldest Schour and Van Dyke group with the most advanced changes in the incisors. Group II was composed of 15 even older animals, those which lived 512 to 664 days after the operation.

Central sections of the incisors were examined for thickening of the labial and the lingual dentin, distortion, folding and degeneration of

enamel epithelium, and other general tissue changes. The type and the frequency of pathologic changes are given in table 2.

Photomicrographs of incisors of normal and hypophysectomized rats may be compared in figures 3 and 4. Figure 3A shows a central section of an upper incisor of a normal rat which had not received any injection and which was 300 days old at autopsy. The blood supply to the pulp and to the paradental structures was abundant. The odontoblasts were

TABLE 2.—*Histologic Analysis of Changes of the Upper Central Incisors\* of Twenty-Six Hypophysectomized Female Rats Following Different Postoperative Periods (Operation at Twenty-Sixth Day of Age)*

Group	Post-operative Period, Days	Age at Autopsy, Days	Stage 1: Thickening of Dentin		Stage 2:	Stage 3:	Stage 4:	Histologic Classification According to Groupings of Schour and Van Dyke	Roentgenographic Analysis According to Table 1: Stages
			Labial	Lingual		Distortion of Apical Curvature Without Folding	Distortion of Apical Curvature With Folding		
1 †	G2295	316		+	—	—	—	I	2
	G7510	323		+	—	—	—	I	2
	B6347	326		+	—	—	—	I	3
	BH8085	338		+	—	—	+	III	4
	GH4607	342		+	—	—	+	II	4
	W3998	345	342-468	+	—	—	+	II	4
	B3856	347		+	—	—	+	III	4
	BH3790	347		+	—	—	+	II	4
	W6679	351		+	—	+	—	I	3
	W3840	376		+	—	—	—	I	2
2	W4120	442		+	—	—	—	I	2
	Total 11 rats.....		11+ = 100%	11+ = 100%	2+ = 18%	5+ = 45%	I 6 = 55% II 3 = 27% III 2 = 19%		
	W1687	512		+	—	+	—	II	4
	W7715	512		+	—	—	—	I	2
	G7113	510		+	—	—	+	II	4
	G6258	534		+	—	—	—	II	2
	W8841	561		+	—	—	—	II	2
	W3762	561		+	—	+	—	I	3
	B4927	574		+	—	—	+	III	4
	B4923	574	538-690	+	—	—	+	III	4
2	W4856	574		+	—	—	—	I	2
	BH01	574		+	—	—	+	III	4
	W56	574		+	—	—	+	II	4
	GH7910	617		+	—	—	+	III	4
	W7650	617		+	—	—	+	III	4
	B3763	645		+	—	—	+	III	4
	G5092	664		+	—	—	—	I	3
	Total 15 rats.....		15+ = 100%	15+ = 100%	3+ = 20%	10+ = 67%	I 4 = 27% II 5 = 33% III 6 = 40%		

\* The lower incisors were not studied histologically.

† This group corresponds to group III of Schour and Van Dyke.

active in the apical half, and labial and lingual dentin were deposited uniformly and stained evenly with hematoxylin-eosin. The pulp canal was wide both in the basal zone and in the central portion of the tooth. The ameloblastic layer was normal in its arrangement and distribution from the apex up to the incisal third of the tooth. Owing to the decalcification process, the enamel which originally covered the convex surface of the central incisor was not seen except in the apical tenth, where the new enamel had contained much organic matter. Enamel epithelium

possessed its normal columnar form throughout the apical two thirds; in the incisal third it was reduced to a thin line of flattened cells. The cementum was approximately 3.75 microns thick. (The latter two structures are present only on the concave or lingual side of the rat's incisor.) The bony crypt envelops the apical four fifths of the tooth and affords firm support.

The severe changes occurring in the rat's incisor after a long post-operative period (617 days, stage 4) are demonstrated in figure 3B. The pulp canal was almost completely obliterated except in the apical fifth. A severe folding of the labial dentin and enamel and a displacement of Hertwig's sheath had taken place. The lingual dentin also showed slight distortion. The cementum was approximately 11 microns thick (three times as thick as the control); this thickening of cementum was observed histologically in all hypophysectomized rats studied. The parodontal bone on the lingual side appeared thin and porous. Enamel formation still continued, though drastically reduced and restricted to the extreme apical region. The few remaining columnar ameloblasts were found only in Hertwig's epithelium sheath. Enamel was absent in the depths of the folds. In these places connective tissue was observed to be in direct contact with dentin. The apical foramen was greatly reduced in size. The tooth was only approximately three fourths the size of the incisor of the control (fig. 3A). It will be noted that the dentin stained much darker after hypophysectomy. This was apparently due to an increase in density.

In contrast to these marked changes, figure 4 demonstrates a central incisor of an animal of the same postoperative period (617 days) with only slight deviation from the normal.<sup>6</sup> Folding was completely absent, changes being restricted to partial obliteration of the pulp canal, the result of thickening of the layers of the labial and the lingual dentin, and degeneration of the enamel epithelium down to the apical third. The parodontal structures were less vascular and more fibrous than those of the normal rat. This incisor was also only three fourths the size of the normal control (fig. 3A).

As the most significant changes were seen at the apex a further analysis of this region is shown in higher magnifications (figs. 5 and 6). Only changes in the upper incisors are illustrated. Since histologic studies were made only of rats living 316 days or more after hypophysectomy, stage 1 could not be demonstrated here.

Figure 5A shows a central section through the apical region of a normal control rat 458 days old. The apical foramen was wide, the

6. The increase in the width of the space at the gingival margins of figures 3B and 4 as compared with that of the control in figure 3A are artefacts produced in histologic preparation.

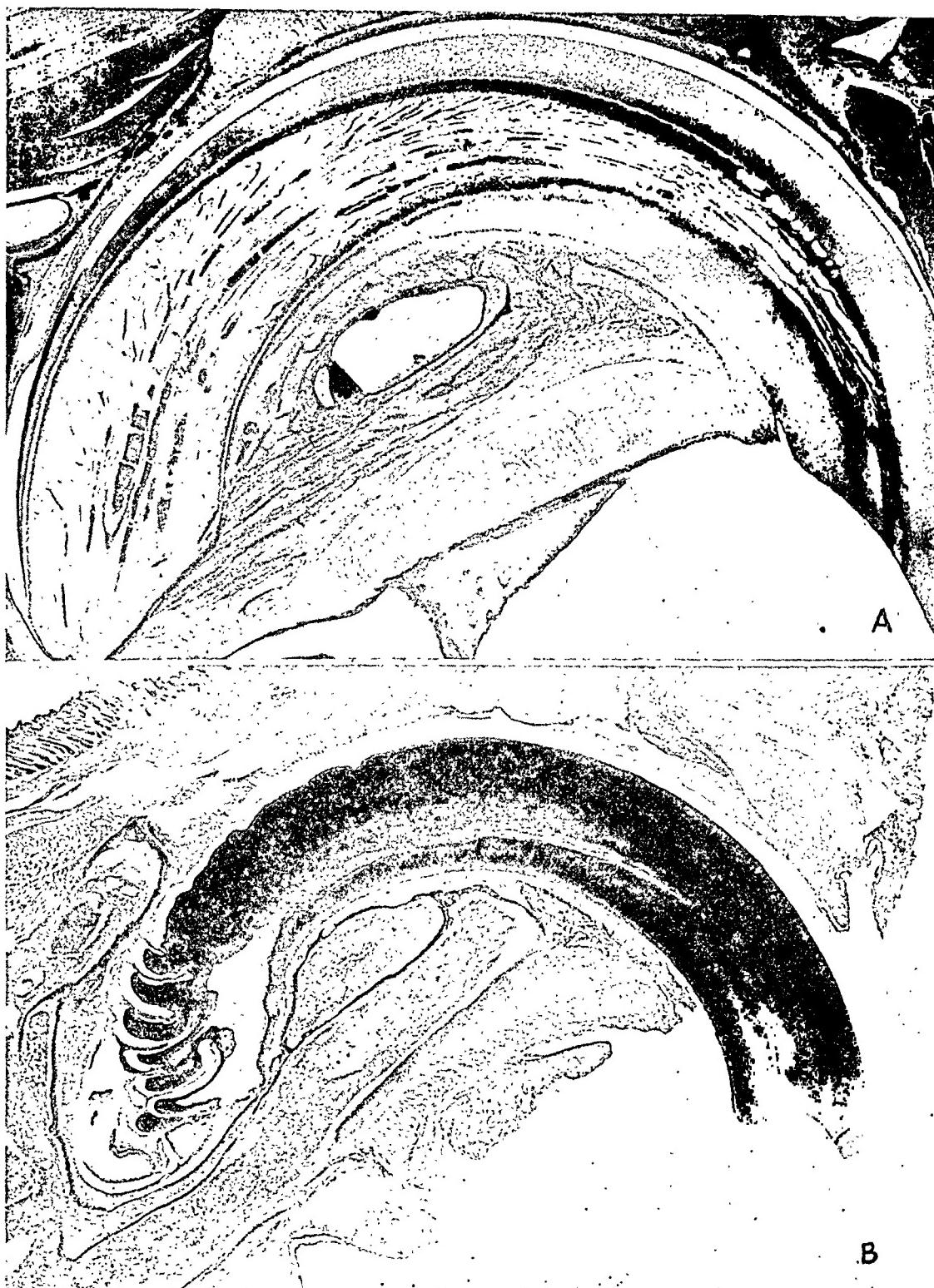


Fig. 3.—A, upper incisor of a normal rat; age at autopsy 300 days. B, upper incisor of a hypophysectomized rat; postoperative period 617 days; age at autopsy 650 days. The pulp canal is almost completely obliterated; there is grotesque displacement of Hertwig's sheath, as well as degeneration of enamel epithelium.

ameloblastic and odontoblastic layers, as well as the enamel and dentin formation, appeared normal. Some enamel matrix could still be seen at the apex. The pulp tissue had an abundant blood supply. Figure 5B shows the apical third of the upper incisor of a hypophysectomized rat which was killed 617 days after hypophysectomy.<sup>7</sup> The apical foramen was distinctly narrower; the vascularity was reduced; the labial dentin was definitely thickened; the lingual dentin, only slightly. It may be seen from this illustration that folding of the hard structures may not occur even after postoperative intervals as long as 617 days.



Fig. 4.—Upper incisor of a hypophysectomized rat; postoperative period 617 days; age at autopsy 650 days. Folding is absent in the apical third. Enamel formation is active. The pulp canal is partially obliterated. The tooth is approximately three fourths the size of the normal control *A* in fig. 3.

Figure 5C demonstrates the much more marked changes, occurring in this instance after a shorter posoperative period than in the preceding case (331 days). Here there was marked thickening of dentin on both the labial and the lingual sides (stage 2). The extreme apical portion of the labial dentin was slightly wavy; the odontoblasts appeared normal. The ameloblasts had degenerated from the incisal third of the tooth to approximately the apical tenth (*a* in fig. 5C). The vascularity of the pulp

7. A higher magnification of the apical region of the incisor is shown in figure 4.



Figure 5  
*(See legend on opposite page)*

and the size of the pulp chamber were reduced to only a fraction of the normal.

The changes characteristic of stage 3 are seen in figure 6A. Thickening of the labial dentin was even more pronounced, and a distinct distortion of the labial curvature at the apex was present. The width of the apex was approximately the same as in the preceding animal. The ameloblasts with normal columnar form were restricted to the apical tenth. The pulp chamber and the vascularity of the pulp were greatly reduced when compared with the normal control of figure 5A.

Figure 6B shows the distortion of labial dentin and enamel and the multiple areas of enamel hypoplasia (stage 4). Folds were seen throughout the apical third. The ameloblasts had degenerated from the incisal region to the apex. There were only a few small islands of columnar ameloblasts between extensive hypoplastic areas. The vascularity of the pulp was severely restricted. The dentin on the lingual side was thick and ended abruptly at the apex instead of tapering as in the normal. The cementum showed increased thickness.

Figure 6C demonstrates the most extreme changes which were observed in the apical region of the upper incisor of the hypophysectomized rat. Labial dentin and enamel were thrown into many overlapping folds. The ameloblasts were flattened or absent. Connective tissue was found in direct contact with the surface of the dentin in the depths of the folds near the apex of the tooth. Hertwig's sheath was markedly displaced, and the apical foramen was reduced to only a small opening. The lingual dentin was not as thickened as in some of the other sections and was irregular in outline. The odontoblasts no longer maintained their orderly distribution as in the normal. They were observed to be shorter and even absent in many areas along the center surface. The cementum was thickened, and the blood supply to the pulp was greatly reduced. The pulp tissue, which is normally very cellular, had become fibrous. The foldings appeared somewhat

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#### EXPLANATION OF FIGURE 5

High magnifications of apical thirds of upper incisors of normal and hypophysectomized female rats: A, normal rat; age at autopsy 458 days. Note the vascularity of the pulp, the extent of the active enamel epithelium and the smooth outlines of the thin labial and lingual dentin. B, hypophysectomized rat; postoperative period 617 days. Note the increased thickness of the labial dentin and the restriction of columnar enamel epithelium to the apical one fifth of the incisor. The thickening of the lingual dentin is not extreme. C, hypophysectomized rat; postoperative period 331 days. Note the definite thickening of the lingual as well as of the labial dentin, typical for stage 2. Restriction of columnar enamel epithelium to the apical tenth of the tooth is observed at a. Note the slight wavy appearance of the apical dentin and enamel on the labial side.

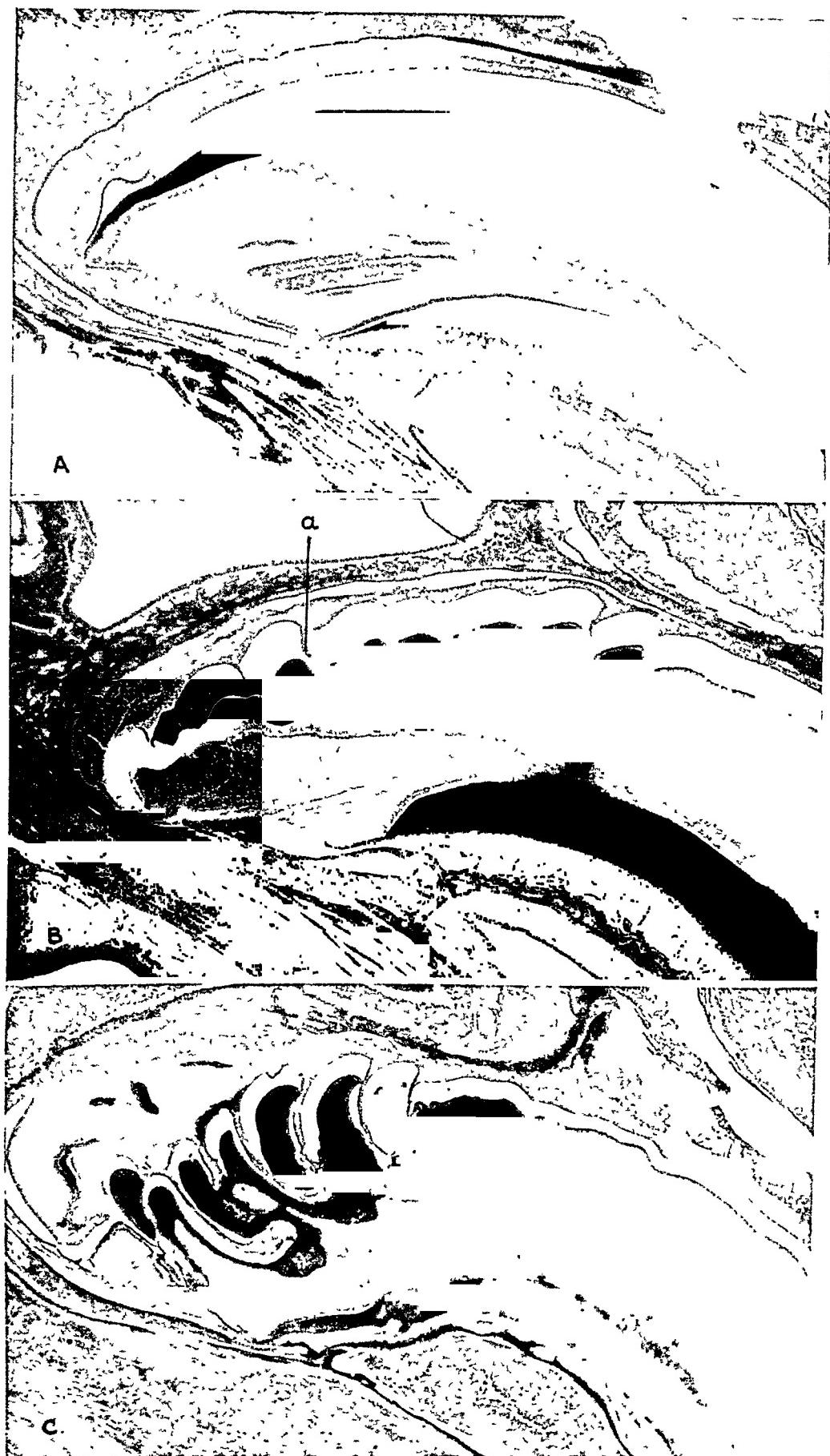


Figure 6  
(See legend on opposite page)

similar to those described for magnesium deficiency of the rat by Becks and Furuta<sup>8</sup>.

A cross section of an upper incisor which yields further information on the nature of the foldings is shown in figure 7. The apical end



Fig. 7.—Cross section through the apical third of an upper incisor of a hypophysectomized rat with severe folding; postoperative period 574 days. Complete degeneration of the enamel epithelium at this level is seen at *A*, and degeneration of odontoblasts at *B*.

of this upper incisor showed roentgenographically marked folding and other characteristics of stage 4. On the labial side the enamel was

8. Becks, H., and Furuta, W. J.: *J. Am. Dent. A.* 28:1083, 1941.

#### EXPLANATION OF FIGURE 6

High magnifications of apical thirds of upper incisors of hypophysectomized rats: *A*, postoperative period 322 days. Note the thickening of the labial and the lingual dentin with distortion of the curvature of the apical tenth typical for stage 3. *B*, postoperative period 345 days. The enamel and the dentin on the labial aspect have a wavy appearance throughout the apical third, found in stage 4. The enamel epithelium has degenerated to the extreme apical end. The projection of the remains of the enamel epithelium and connective tissue at *a* indicates hypoplasia. The vascularity and the cellular content of the pulp are greatly reduced. *C*, postoperative period 617 days. There is severe folding of labial tissues, typical for stage 4, with extreme displacement of Hertwig's sheath. Note the severe hypoplasia of the enamel, with the surface of the dentin in direct contact with the connective tissue. Some enamel epithelium is still present in the displaced Hertwig's sheath. The vascularity and the cellular content of the pulp are greatly reduced, as in figure 6*B*.

still present (*A* in fig. 7); however, the ameloblastic layer had completely degenerated. The odontoblasts had also disappeared for the main part (*B* in fig. 7). The dentin was seen to fold not only in the labiolingual direction but in the mesiodistal direction.

#### COMMENT

The roentgenographic study of the teeth from 141 hypophysectomized rats has disclosed no detectable change up to 12 days after operation. The first morphologic deviations from the normal were noted in the incisor 14 days after operation and consisted of thickening of the labial dentin layer (mantel dentin). This was especially noticeable in the posterior half of the tooth and increased progressively with longer postoperative periods. This increase in thickness of dentin led to gradual and in many instances complete obliteration of the pulp. These changes are due not only to disturbance in tooth formation but to retardation in the rate of eruption.<sup>9</sup> Since 63 post-operative days was the shortest period reported by Schour and Van Dyke, the early stages were not described by them.

Thickening of the lingual dentin was seen for the first time 87 days after the operation, but since the next shorter period studied was 43 days, this stage may begin earlier than 87 days. By this time, the labial dentin was markedly thickened, and obliteration of the anterior half of the pulp chamber had been completed. This lingual thickening was characteristic of all animals with longer postoperative intervals. Furthermore, it can be stated that the changes described as characterizing stages 1 and 2 were the only ones which occurred consistently in every hypophysectomized rat after the time of their appearance, 14 and 87 days, respectively. From this it may be said that the thickening of the labial and the lingual dentin and the smaller size of the tooth are the only consistent pathognomonic signs of hypophysectomy which were recognized in the roentgenograms.

The changes which were characteristic of stages 3, 4, and 5, i.e., distortion of the apical curvature without folding, folding of the apical third of the upper incisor without folding of the lower incisor, and finally folding of the posterior portion of both the upper and the lower incisor, are by no means consistent or uniform and therefore are not pathognomonic for hypophysectomy. For instance, after a period of 100 days or more a distortion of the apical curvature without folding occurred only in 15 of 93 animals, i.e., in 16 per cent (table 1). This distortion represents the forerunner of the foldings seen in stages 4 and 5. It was an abrupt change in the outline of the smooth convex

9. Studies of eruption rates in hypophysectomized female rats will be reported separately.

surface of the incisor where the apical end dropped off to form an acute angle with the other portion of the tooth. It could be detected roentgenographically in every instance in which it occurred because of the characteristic deviation from the normal outline of the surface. One gains the impression from histologic observation that this change in curvature at the apical end is an advanced expression of a disproportion in the rate of the eruption and the rate of the active formation of the tooth. The earliest expression of this disproportion was thickening of the labial and the lingual dentin.

The folding of the apical region described as stage 4 (table 1) was also inconstant. It was observed in only 40 of 86 (46 per cent) of the hypophysectomized female rats 144 and 664 days after the operation. In rats which lived from 144 to 210 days after the operation this folding was even less frequent; it was seen in only 7 of 26 animals (27 per cent).

Since Schour and Van Dyke concluded that folding of the apical region of the incisor is a feature pathognomonic of hypophysectomy in rats which survived a period of 6 months and longer and since folding was not found to be a constant characteristic even at much longer postoperative periods, it appeared important to analyze further the incidence of these advanced pathologic changes. The distribution of the animals of Schour and Van Dyke (groups I, II and III) as compared with the animals described in this report is indicated in the last column of table 1.

In comparing the frequency of changes reported by Schour and Van Dyke, based on 23 rats, with that found for the larger group of 71 rats with comparable postoperative intervals used here, the following observations were made:

Among the 17 rats that lived from 87 to 144 days after hypophysectomy (corresponding to group I in the report of Schour and Van Dyke) only 1 animal was found with definite folding of the labial hard structures in the upper incisor (stage 4) yet with no folding of the lower incisor. This represented only approximately 5 per cent of the group of 17 animals. The frequency of folding increased to 30 per cent of the 26 animals which survived 205 to 302 days (corresponding to group II of Schour and Van Dyke), and to approximately 50 per cent of the 28 animals which survived 316 to 442 days (corresponding to their group III).

Schour and Van Dyke also differentiated between various degrees of folding and described folding of the enamel epithelium with definite indentations in the enamel surface as characteristic of their group I (8 rats). In this study such indentations in the enamel were not observed in the group of 17 animals of comparable postoperative age, with the exception of 1 with a definite folding of the apex. Schour and

Van Dyke also demonstrated the folding of the lower central incisor as characteristic of animals in their group II. Among the corresponding 26 rats of this report, only 2 (approximately 8 per cent) showed a folding of the lower incisor. In the age group corresponding to group III of Schour and Van Dyke (316 days or more) the frequency of the labial folding of the lower incisor increased to approximately 15 per cent; these changes did not always occur simultaneously with the folding in the upper incisor.

In this series studies were made of 28 hypophysectomized rats which survived up to 222 days longer than any of those reported by Schour and Van Dyke. Even for this older group it was not possible to state that the advanced changes at the apical end of the upper or the lower incisor were either constant or pathognomonic for complete hypophysectomy of the rat.

Since only rats with the longest postoperative periods were studied histologically, the early stages, 1 and 2, the thickening of labial and lingual dentin, were found in each specimen. As may be seen from table 2, stage 3, the distortion of apical curvature without folding, was observed only twice (18 per cent) in group I, which corresponds to group III of Schour and Van Dyke as far as postoperative periods are concerned. Stage 4, the most extreme distortion of the apical curvature with folding, was seen only five times (45 per cent). These percentages increased slightly in group II, with postoperative periods of from 512 to 664 days, to 20 per cent and 67 per cent, respectively. In checking the histologic changes against the groupings of Schour and Van Dyke, the interesting picture is revealed (table 2) that their group III, with the most extreme changes, is represented only twice (19 per cent) in our group I, group II three times (27 per cent) and group I six times (55 per cent). Even after longer postoperative periods (table 2, group II) group III of Schour and Van Dyke is found only six times (40 per cent), group II five times (33 per cent) and group I four times (27 per cent). These percentage figures check closely with the roentgenographic analysis (table 1).

It is possible that the greater physical vigor of the strain of rats used in this experiment and the hygienic conditions may account for the late appearance and the lower frequency of the foldings observed in this study.

In comparing the histologic observations of the teeth of 26 animals with the roentgenographic observations (last column in table 2) it was found that with the exception of the earliest displacement of Hertwig's sheath, which is not visible in the roentgenogram, all histologic changes confirmed the roentgenographic classification. This exception was noticed in rat W3762 (table 2, group II).

## CONCLUSIONS AND SUMMARY

Roentgenographic and histologic studies of the incisors of 141 female hypophysectomized rats, killed at intervals between 6 and 664 days after the operation, permit the following conclusions:

The earliest change in the upper incisor as seen in the roentgenogram was thickening of the labial and the lingual dentin at the expense of the pulp chamber. This alteration occurred at 14 and 87 days, respectively, after hypophysectomy and was the only constant and pathognomonic symptom observed.

Distortion of the apical labial curvature, distinct folding of the upper incisor alone, and folding of both upper and lower incisors were first seen 100, 144 and 205 days, respectively, after hypophysectomy. None of these symptoms was pathognomonic. The folding of the upper incisors alone was most frequently seen. It occurred in 46 per cent of the hypophysectomized rats 144 or more days after the operation. Folding of the lower incisors was present in only 13 of the 40 rats which showed folding of the upper incisors. It never occurred alone, i.e., without simultaneous folding in the upper jaw. The various degrees of folding were found to be an expression of delays in eruption.

Histologic observations were limited to animals with long post-operative intervals. The most outstanding change in cellular structures occurred in the ameloblastic layer. The regressive changes were not uniform for all animals but were directly proportionate to the severity of the distortion or folding of the apical tissues. Degeneration of the ameloblasts in localized areas led to hypoplasia of the enamel and in some places to agenesis of the enamel so severe as to leave connective tissue in direct contact with the surface of the dentin.

Degeneration of the odontoblasts began in the incisal portion of the incisor and reached the apical end only in animals that showed advanced folding. Marked diminution in the vascularity of the pulp also characterized the advanced changes.

Increase in the thickness of the cementum was observed histologically in the incisors of all hypophysectomized rats and did not vary with the postoperative interval (316 to 644 days after the operation).

## RENAL LESIONS IN PORTAL CIRRHOSIS

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PATHOLOGIC changes occurring simultaneously in the liver and the kidneys have been described in a number of disease processes. Heyd,<sup>1</sup> in his classification of "liver deaths," emphasized the occurrence of progressive signs of renal failure culminating in anuria and death. This syndrome has become widely known as the hepatorenal syndrome. Wilensky,<sup>2</sup> Reich<sup>3</sup> and Boyce<sup>4</sup> have reviewed the phenomenon at length. Symptoms of renal failure are frequently seen along with evidence of hepatic disease following use of toxic drugs and chemicals, burns, intestinal obstruction, toxemia of pregnancy, infectious disease, such as Weil's disease and yellow fever, and other conditions. Orr and Helwig<sup>5</sup> emphasized the importance of renal failure following traumatic injury of the liver.

The majority of workers have thought the renal failure to be due to toxic substances acting on the tubular epithelium. These substances are assumed to arise as products of chronic infection, metabolism, or breakdown of liver cells or other tissue and to reach the kidneys in sufficient amounts to cause damage because they are not detoxified by the poorly functioning liver. Lichtman and Sohval,<sup>6</sup> on the other hand, attributed the anuria and the azotemia occurring in hepatic disease to prerenal deviation of fluids.

In cases of the hepatorenal syndrome of the type described by Heyd the pathologic changes of the kidneys have not always corresponded in severity to the clinical manifestations. Although it has been stated that tubular degeneration occurs, there have been few extensive and detailed descriptions of the renal changes. Renal lesions resulting from the action of certain chemical agents which also produce hepatic injury have been

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1. Heyd, C. G.: Am. J. Digest. Dis. **9**:348, 1942.

2. Wilensky, A. O.: Arch. Surg. **38**:625, 1939.

3. Reich, N. E.: Internat. Clin. **4**:135, 1941.

4. Boyce, F. F.: The Role of the Liver in Surgery, Springfield, Ill., Charles C Thomas, Publisher, 1941.

5. Orr, T. G., and Helwig, F. C.: Ann. Surg. **110**:682, 1939.

6. Lichtman, S. S., and Sohval, A. R.: Am. J. Digest. Dis. **4**:26, 1937.

fairly extensively studied. Lichtman and Sohval<sup>6</sup> described granular, hydropic, vacuolar and fatty degeneration in the convoluted tubules of the kidney in 12 cases of acute and subacute yellow atrophy of the liver. Presumably most or all of the patients were jaundiced, and the renal lesions might be attributed, at least in part, to this factor.

Anatomic changes in the kidney, consisting chiefly of tubular degeneration, resulting in disturbances of renal function and abnormal urinary findings, have been frequently described in marked jaundice.<sup>7</sup> This process has been called bile or cholemic nephrosis. Stewart and Cantarow<sup>8</sup> noted the occurrence of degenerative changes in the renal tubular epithelium in cats and dogs following the administration of relatively small amounts of sodium dehydrocholate. Regeneration of the tubular epithelium occurred in a few days. The effect of naturally occurring bile acids was not determined. Whether renal damage might be produced by excessive urinary excretion of bile acids in cases of liver disease, even without jaundice, has not been definitely determined but does not appear likely to us.

Symptoms of renal failure and renal lesions occurring in cirrhosis of the liver have not been widely emphasized. Albuminuria, abnormal urinary sediment and azotemia have been reported even in the absence of jaundice,<sup>9</sup> but we know of no detailed studies of the anatomic changes in the kidneys of patients with cirrhosis of the liver.

In recent years, studies of the effects on experimental animals of diets deficient in certain substances have thrown additional light on the relationship of degenerative changes in the liver and the kidneys. Following the observations of Allen and associates<sup>10</sup> and Fisher<sup>11</sup> in 1924 that in depancreatized dogs treated with insulin fatty livers developed, Best and associates<sup>12</sup> found that the fatty changes could be prevented by choline. It was observed that casein (containing methionine),<sup>13</sup> methionine<sup>14</sup> and certain other substances were lipotropic, and this was explained by du Vigneaud<sup>15</sup> as due to the ability

7. Elsom, K. A.: Arch. Int. Med. **60**:1028, 1937. Thompson, L. L.; Frazier, W. D., and Ravdin, J. S.: Am. J. M. Sc. **199**:305, 1940.

8. Stewart, H. L., and Cantarow, A.: Arch. Path. **20**:866, 1935

9. Lichtman, S. S.: Diseases of the Liver, Gallbladder and Bile Ducts, Philadelphia, Lea & Febiger, 1942.

10. Allen, F. N.; Bowie, D. J.; McLeod, J. J. R., and Robinson, W. L.: Brit. J. Exper. Path. **5**:75, 1924

11. Fisher, N. F.: Am. J. Physiol. **67**:634, 1924.

12. Hershey, J. M.: Am. J. Physiol. **93**:657, 1930. Best, C. H.; Hershey, J. M., and Huntsman, M. E.: J. Physiol. **75**:56, 1932. Best, C. H.; Ferguson, G. C., and Hershey, J. M.: ibid. **79**:94, 1933

13. Best, C. H., and Huntsman, M. E.: J. Physiol. **83**:255, 1935.

14. Tucker, H. F., and Eckstein, H. C.: J. Biol. Chem. **121**:479, 1937.

15. du Vigneaud, V.: Biol. Symposia **5**:234, 1941.

of these substances to furnish labile methyl groups for the synthesis of choline. György<sup>16</sup> recently reviewed the subject of the production of hepatic injury and cirrhosis by dietary means.

In 1939 Griffith and Wade<sup>17</sup> noted the occurrence of renal tubular degeneration with cortical congestion and hemorrhages within six to eight days in young rats fed diets deficient in choline and other lipotropic factors. Impairment of renal function and albuminuria were also noted in these animals. Renal lesions produced in rats by choline deficiency were further studied by Christensen.<sup>18</sup>

Since, more and more, a role of deficiency of lipotropic substances in degenerative disease of the liver in man is being suspected,<sup>19</sup> it seemed to us that renal lesions due to the same deficiencies and similar to those produced in the rat by choline deficiency might be found in these cases. One might expect that the renal lesions would be more prominent in the cases of acute disease, that is in acute and subacute necrosis of the liver. However, since nearly all of these patients are jaundiced, and since tubular degeneration has been attributed to jaundice, we decided to study the renal changes in cases of portal cirrhosis, many of which are not complicated by jaundice.

#### METHOD OF STUDY

Twenty-five cases of portal cirrhosis were selected from the autopsy material of the last ten years at Parkland Hospital. Certain cases were excluded from the series because of complicating renal disease of an inflammatory or a vascular nature. Others were discarded because of severe infection, since it is known that tubular degeneration occurs under this circumstance. Instances of obstruction of the biliary system were also excluded.

Portal cirrhosis was recognized on the basis of nodular regeneration of the hepatic parenchyma accompanied by proliferation of periportal fibrous tissue. Twenty-two of the cases were considered to be of the Laennec type, while 3 were classified as instances of toxic cirrhosis because of evidence of recent or old extensive hepatic necrosis. In most cases the cirrhosis was recognized grossly without difficulty, but in a few cases nodular regeneration was slight and identified with certainty only by microscopic study.

In each case sections of liver and kidneys stained with hematoxylin-eosin were studied microscopically. In 9 cases sudan IV stain for fat, Mallory's aniline blue connective tissue stain and congo red stain were utilized. Clinical records and autopsy protocols were studied for accessory data.

#### CLINICAL AND GROSS PATHOLOGIC DATA

Many of the patients were admitted in coma or following massive hemorrhage, and it was often impossible to obtain adequate historical data. Most of the patients had had signs or symptoms pointing toward the diagnosis of cirrhosis for from

16. György, P.: Am. J. Clin. Path. **14**: 67, 1944.

17. Griffith, W. H., and Wade, N. J.: J. Biol. Chem. **131**: 567, 1939.

18. Christensen, K.: Arch. Path. **34**: 633, 1942.

19. Russakoff, A. H., and Blumberg, H.: Ann. Int. Med. **21**: 848, 1944.

two days to five years. Nine patients had jaundice which was of moderate intensity in most of them, but icterus indexes were distributed from 10 to 200. Sixteen had ascites. Eight patients had massive bleeding from esophageal varices, and 7 died from this cause. Fifteen apparently died in coma, presumably as a result of hepatic insufficiency, whereas the remaining 3 died from miscellaneous causes unrelated to the cirrhosis.

Serologic tests for syphilis were positive in 5 of 23 cases. Blood urea was determined in 9 cases, and in only 1 was it normal; in 1 case of cirrhosis with superimposed acute necrosis it was decreased, and in the remaining 7 cases it was slightly to moderately elevated. The level of total serum proteins was determined in 10 cases and was revealed to be subnormal in the majority, and in 5 of these cases the albumin-globulin ratio was definitely inverted. Urinalyses, performed in 20 cases, showed albuminuria present in 60 per cent.

No detailed dietary histories were available. Five of the patients were known to have had chronic alcoholism. Three had received arsenical therapy for syphilis, and 3 received mercurial diuretics without evident toxic reactions.

In the majority of cases the gross characteristics of the liver were typical of portal cirrhosis. Five livers weighed less than 1,000 Gm. Ten weighed 1,000 to 1,500 Gm., 5 between 1,500 and 2,000 Gm., and 3 weighed more than 2,000 Gm. Three showed marked fatty changes, and in 3 cirrhosis was complicated by primary carcinoma.

The spleen in most cases was slightly to moderately enlarged. Two of the spleens were small, weighing less than 100 Gm. Nine weighed between 130 and 180 Gm. and 12 between 215 and 650 Gm.

In nearly all cases the kidneys appeared essentially normal. In 2 cases the individual kidneys weighed a little more than 200 Gm. In a few instances the cortex was slightly swollen and pale. Some of the kidneys revealed fine granularity after stripping of the capsule, presumably due to patchy areas of dilated or hypertrophied tubules, and an occasional kidney had small depressed cortical scars. The ureter and the pelvis of 1 kidney were dilated without obvious cause, and the pelvis of 1 kidney contained a small stone, without obstruction.

Five patients were found at autopsy to have mild hypostatic bronchopneumonia. Three patients had primary carcinoma of the liver, 2 had bronchogenic carcinoma and 4 patients had carcinoma of the following regions, respectively, gallbladder, esophagus, breast and face (epidermoid, squamous cell type). In none of these was the additional pathologic process considered to play a significant part in the illness or the death of the patient.

#### HISTOLOGIC OBSERVATIONS

*Glomerular Lesions.*—Minor alterations in the glomeruli were common in this series. In 19 cases there were scattered, completely hyalinized glomeruli, occurring in areas without other significant sclerotic changes. In 10 cases there were changes described as periglomerular fibrosis. This consisted of actual fibrous tissue proliferation and elaboration of collagenous intercellular material outside the basement membrane. Often this process was localized to one side of the glomerulus, but usually a complete ring of fibrous tissue extended around the glomerulus, which was itself often atrophic. In most of these altered glomeruli there was also hyaline thickening of the parietal and occasionally of the visceral basement membrane. In every case in which there was periglomerular fibrosis there was also some degree of arterial or arteriolar sclerosis. Because of this association it seems likely that periglomerular fibrosis and hyaline thickening of the glomerular

basement membrane were due to arterial and arteriolar sclerosis, thus resembling the ischemic changes in the glomeruli described by Kimmelstiel.<sup>20</sup> Furthermore, there was no apparent correlation between the occurrence or severity of the glomerular changes and the tubular degeneration, atrophy and regeneration noted in the following section. It is of some interest, however, that Christensen<sup>18</sup> described periglomerular fibrosis and thickening of the basement membrane of the glomeruli in rats which had been fed diets deficient in choline.

In many cases in which there was marked tubular degeneration the glomerular spaces contained pink protein precipitate.

*Tubular Changes.*—1. Types and Incidence of Lesions Encountered: The most common lesion was granular swelling (fig. 1A) of the convoluted tubules. This was noted in some degree in every case but was quite prominent in 18 of the 25. In the other 7 cases either slight diffuse or focal swelling of epithelial cells was revealed. Other tubular changes, consisting of atrophy, dilatation and regeneration, occasionally masked the appearance of the granular swelling. The proximal tubules were most strikingly involved. Henle's loops and the distal convoluted tubules showed similar but much less marked alteration. The process was focal or diffuse and consisted of marked enlargement of the cell bodies, with prominence of cell outlines and cloudiness or fine to coarse granularity of the cytoplasm. The granularity was often uniform throughout the cell body, but in some instances the granules at the tips of the cells were sharper and larger. The tips of the cells and usually the brush borders were well preserved. The lesion described here was indistinguishable from that which is well known as acute parenchymatous degeneration or cloudy swelling.

In 10 cases there was also hydropic swelling of the cells of the proximal convoluted tubules. This occurred in those cases in which granular swelling was marked. It consisted of a greater degree of enlargement of the cells with the appearance of clear areas where the granules appeared to be separated from one another by watery cytoplasm.

In 7 cases small vacuoles were noted in the proximal convoluted tubules. Hydropic swelling was present in all these 7 cases except 1. The vacuoles were most numerous at the bases of the cells but occurred to some extent over the entire bodies of the cells, giving them a foamy appearance. This vacuolation was often marked in the straight segments of the convoluted tubules. Fat stains demonstrated the vacuoles to be fat droplets in most instances, but in some areas the vacuoles were fat-free. With this stain, fat droplets were found to be most numerous at the bases of the cells, where they were often arranged in fine parallel rows extending longitudinally in the cells. Fat droplets were less frequently noted in the cells lining Henle's loops, and here they tended to be larger and less numerous than in the convoluted tubules.

Hyaline droplets (fig. 1B) were found in the proximal convoluted tubules in 5 cases. They occurred in focal groups of tubules of the same nephron unit. The droplets were located mainly in the tips of the cells, were brightly eosinophilic and hyaline in structure and did not stain with congo red. There appeared to be no correlation between the occurrence of hyaline droplets and the severity of other tubular changes.

In 10 cases there was dilatation of the convoluted tubules, with the lining being converted to a thin, atrophic-appearing epithelium (fig. 1C). In the most characteristic form these dilated tubules were found scattered throughout the cortex in focal areas involving several tubular loops of the same nephron unit. The out-

side diameter of these tubules was increased, but the inside diameter was even more increased because of the thinning of the lining cells. These dilated tubules occasionally enclosed hyaline casts or protein-containing fluid. In a few cases dilatation of the convoluted tubules was diffuse and was not so marked as in the focal lesions. It appeared to be almost entirely due to atrophy of the lining cells with widening of the lumen.

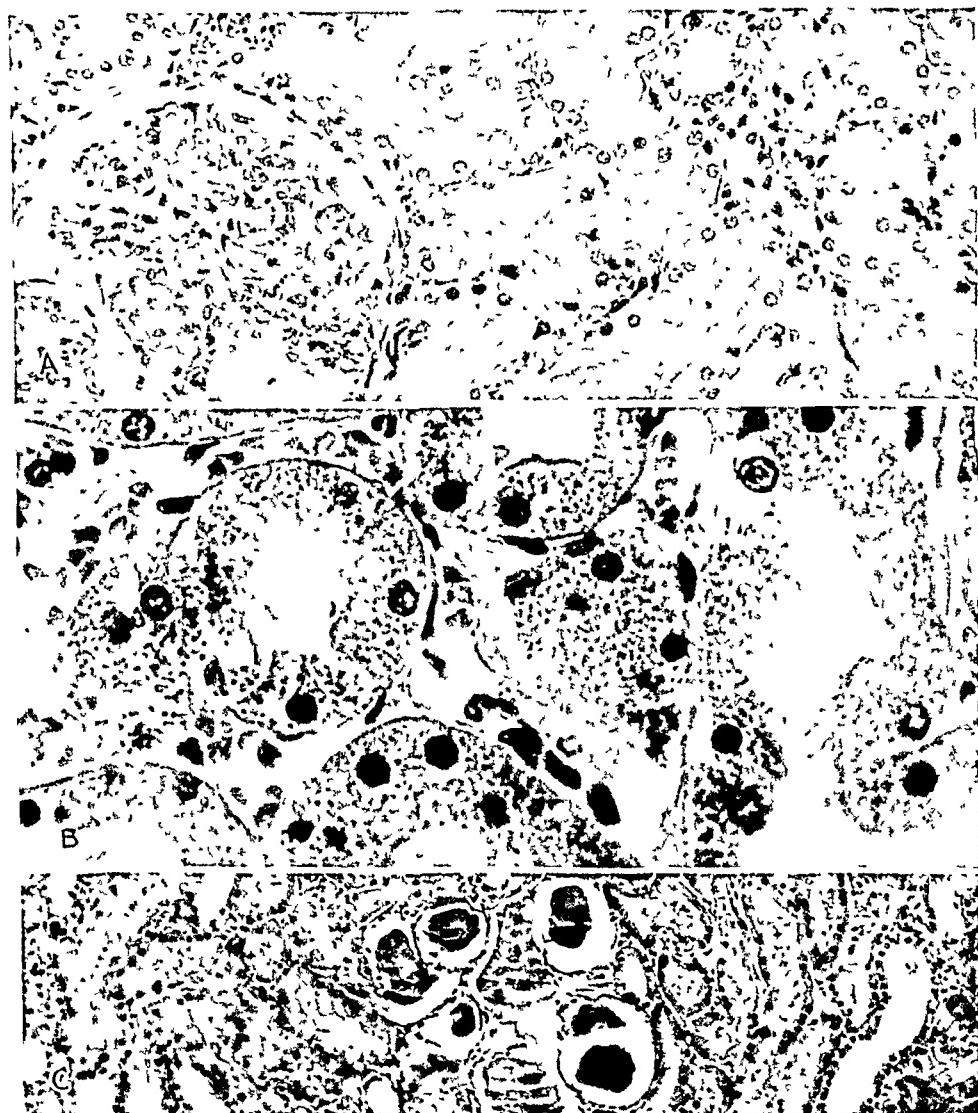


Fig. 1.—*A*, section of kidney showing granular swelling of the epithelium of convoluted tubules and periglomerular fibrosis.

*B*, hyaline droplet formation in swollen epithelial cells of convoluted tubules.

*C*, section of kidney revealing focal granular swelling, dilatation and epithelial atrophy and, in addition, regeneration of epithelial cells of convoluted tubules. Hyaline casts are seen in dilated tubules.

In close association with dilatation and atrophy, 7 cases revealed certain changes in the convoluted tubules which seemed to indicate regeneration of the epithelial cells (fig. 2 *A* and *B*). Some dilated tubules appeared to be denuded of epithelium.

Others were lined by low columnar or cuboidal epithelium in which the nuclei were hypertrophied and hyperchromatic. The nuclei were also more numerous in these tubules, and mitoses were observed occasionally. In some instances there were scattered multinucleated syncytial masses in the epithelial lining. The cytoplasm of these regenerating cells was scanty and slightly basophilic. In addition to the dilated tubules with regeneration of epithelium there were frequent groups of tubules in the cortex in which the lumens were small and collapsed, which appeared to be lined by regenerating elements similar to those just described.

No consistent changes were noticed in the interstitial tissue of these kidneys. The collecting tubules were occasionally somewhat dilated, particularly in focal areas, but were otherwise unaltered.

2. Pattern and Possible Evolution of Renal Lesion: With respect to the renal changes, these cases would seem to fall into two general histologic groups. In one group, including the majority of cases, the picture was that of acute tubular injury, consisting of granular swelling, hydropic degeneration, fatty and vacuolar degeneration and presence of hyaline droplets in the convoluted tubules. These changes occurred focally or diffusely and in variable degrees of intensity. In this group the changes were thus characteristic of the acute tubular degenerations sometimes referred to as the acute nephroses.

In the other group the renal changes were of a more chronic nature. The degenerative phenomena were present but less marked, and the predominating lesion consisted, instead, of focal or diffuse dilatation of the convoluted tubules. This was accompanied by atrophy and evidence of regeneration of the tubular epithelium. These tubular changes are indicative of a longer-standing tubular injury with added reparative processes.

Analysis of the various lesions of the kidney revealed what appeared to be a transition from the acute type of tubular swelling to the chronic tubular lesions just described. Many kidneys showed the two processes occurring simultaneously in different areas. Where tubular dilatation with atrophic epithelium was extensive, the acute degenerative lesions were not marked. Regenerative epithelium seemed to replace previously degenerated epithelial cells. It is believed that the first stage of renal tubular injury is that of acute swelling with or without hydropic, vacuolar and fatty degeneration. Following this stage three courses might conceivably be followed: First, there might be complete regression of the degenerative phenomena with return to a normal morphologic state; second, cytoplasm might be partially lost into the lumen from the swollen cells, followed by a state of progressive restitution by cytoplasmic regeneration; or, finally, the cells might undergo necrosis, be removed by disintegrating into the lumen or by autolysis, and in turn, be replaced by regenerating epithelial cells. The morphologic characteristics of the second and the third course are not distinguished with certainty at present.

#### CORRELATION OF ACTIVITY OF CIRRHOSIS AND SEVERITY OF RENAL LESIONS

An attempt was made to grade the activity of the cirrhotic process in each case, grades of from 1 to 4 plus being used and degenerative changes in the parenchymal cells, fatty infiltration, regeneration, fibrosis and lymphocytic infiltration being taken into consideration as criteria (fig. 2 C and D).

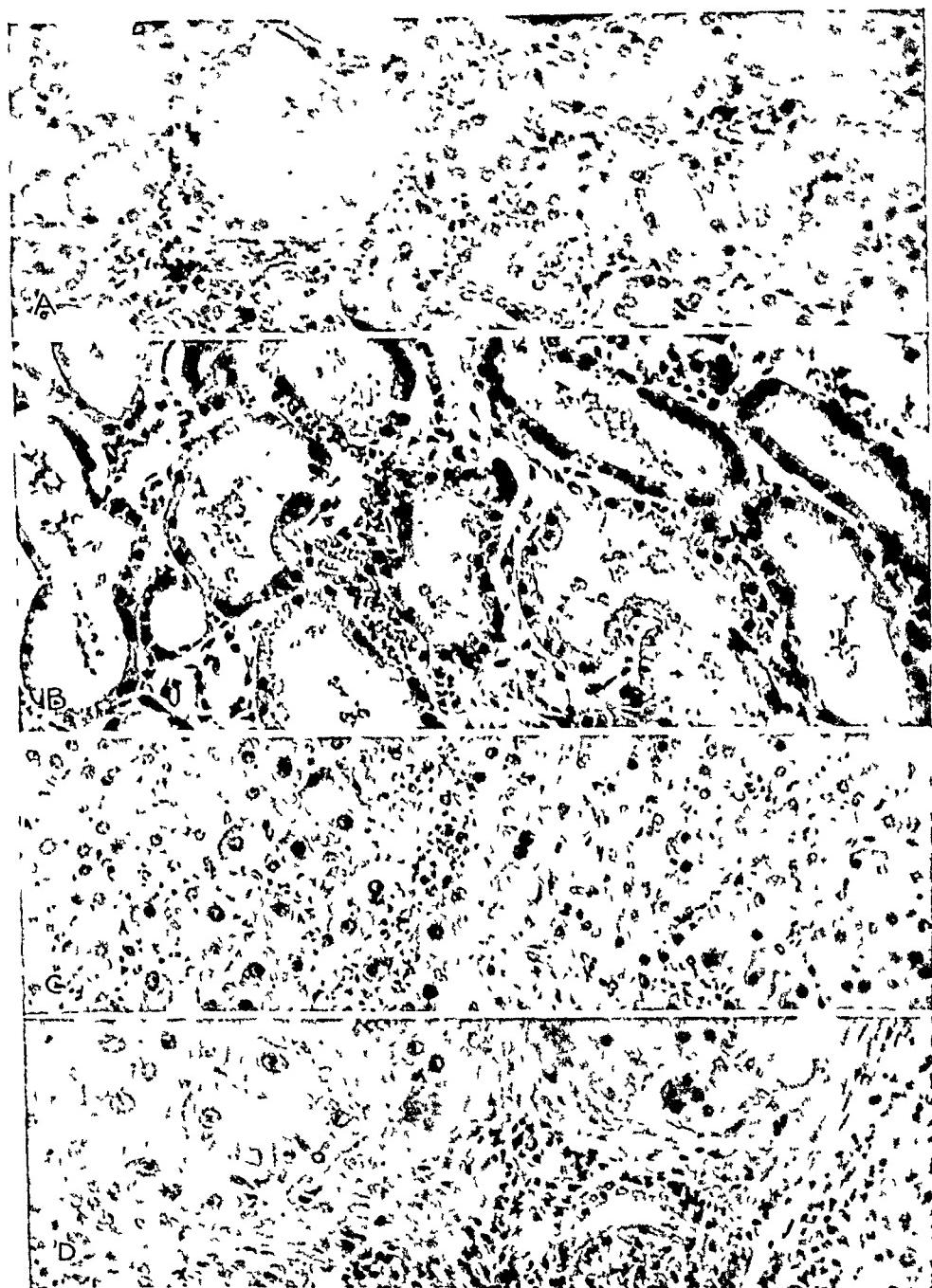


Fig. 2.—*A*, focal tubular dilatation. Note the markedly dilated tubule lined partly by granular swollen cells and partly by flattened cells. Regeneration of epithelium is prominent in the small tubules.

*B*, marked diffuse dilatation with flattening of lining cells and some evidence of regeneration of the epithelium of the convoluted tubules.

*C*, cirrhosis of the liver showing slight degree of activity as far as regenerative and inflammatory changes are concerned.

*D*, cirrhosis of the liver with markedly active regeneration, considerable lymphocytic infiltration and focal fatty metamorphosis. A moderately high degree of activity of cirrhosis is seen.

The severity of the renal changes in each case was graded in a similar manner without reference to the grade of activity of the cirrhosis. In grading the severity of the renal changes the degree of granular swelling of the epithelial cells of the convoluted tubules was regarded as the most important criterion. However, tubular atrophy, dilatation and regeneration, vacuolation of tubular cells, hydropic degeneration and hyaline droplet formation were also noted, and when these changes were present in a significant degree the grade of the granular swelling was increased to obtain the final grade of the renal lesions.

In order to determine whether there is any correlation between the activity of the cirrhotic process and the severity of the renal lesions, a

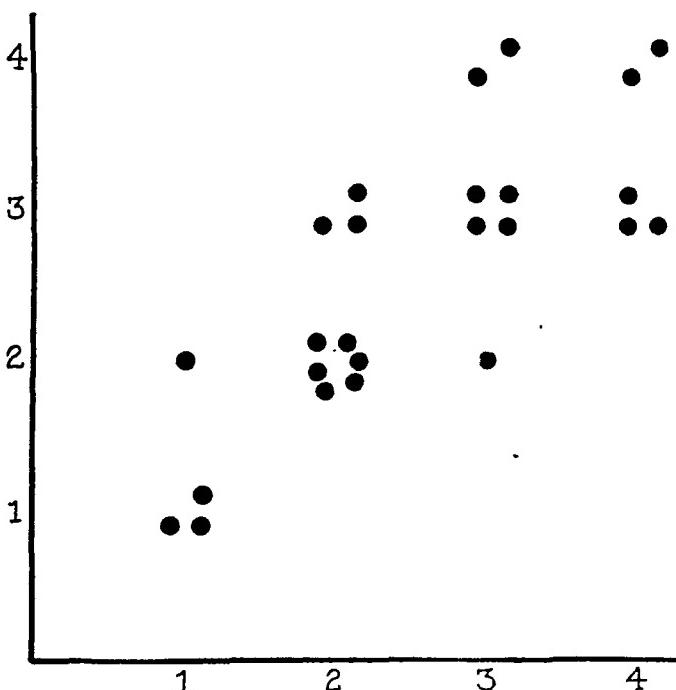


Fig. 3.—Scattergram showing the correlation between activity of cirrhosis (grades 1 to 4 indicated at bottom of chart) and severity of tubular changes (grades 1 to 4 indicated at left).

scattergram was made (fig. 3), the activity of the cirrhosis being used as abscissa and the severity of the renal lesions as ordinate. It can be seen from the graph that there is a definite correlation between the two functions; that is, the more active the cirrhosis, the more severe are the renal lesions. This would seem to provide further evidence that the renal lesions encountered in these cases are related in some way to the cirrhosis itself.

#### RELATIONSHIP OF JAUNDICE TO THE RENAL LESIONS

Since renal tubular degenerative changes are known to occur in jaundice, supposedly due to the effect of bile salts on the epithelial cells,

it should be considered whether the renal lesions in these cases are to any extent dependent on the presence of jaundice. Table 1 shows the number of cases with each grade of tubular degeneration among the cases of cirrhosis with and the cases without jaundice. It will be noted that even the severer grades of tubular degeneration are seen in the cases without jaundice as well as in those with jaundice. However, the average grade of tubular degeneration is greater in the cases with jaundice. This does not necessarily mean that jaundice per se is even partially responsible for the renal changes, however. Since the activity of the cirrhosis is in general greater in the cases with jaundice, it may be that the extent and the severity of the hepatic disease determine the magnitude of the renal changes. The extent of the renal changes seems to be in proportion to the activity of the cirrhosis irrespective of the presence or the absence of jaundice.

#### COMMENT

*Causes of the Tubular Lesions.*—The various degenerative lesions which have been observed in the convoluted tubules and loops of Henle

TABLE 1.—Relationship of Jaundice to Tubular Degeneration

Cases	Cases with Given Grade of Tubular Degeneration			
	1	2	3	4
Cirrhosis with jaundice.....	9	0	1	6
Cirrhosis without jaundice.....	16	3	7	1

appear to be related in some way to the cirrhosis. Disease processes unrelated to cirrhosis can be excluded as a causative factor in most cases of the series. The possibility that jaundice plays an important part appears unlikely. Doubt has already been expressed that tubular injury might be produced even in cases without jaundice by excessive elimination of bile acids in the urine in cirrhosis. Renal anoxia has been suggested as a cause of the tubular degeneration and impairment of renal function occurring in a variety of conditions,<sup>21</sup> and it cannot be entirely eliminated as a possible factor in the production of the lesions observed in some of the cases of the present study.

In view of the fact that Griffith and Wade<sup>17</sup> and Christensen<sup>18</sup> have described similar tubular degeneration occurring simultaneously with hepatic injury in the rat under conditions of experimental choline deficiency, it seems to us that the tubular degeneration observed in this series of cases might be explained on the basis of a similar mechanism; that is, the renal injury is produced by the same factors which produce

21. Macgrath, B. G.; Havard, R. E., and Parsons, D. S.: Lancet 2:293, 1945.

the hepatic injury. It is difficult or impossible to prove even in animals that the renal damage is a result of the factors which cause the hepatic injury rather than a result of the hepatic injury itself. Dietary deficiency of lipotropic substances and circumstances which increased the requirement of or interference with the action of lipotropic substances seem to be important factors but are not the only ones which play a part in the production of cirrhosis and injury of the liver.<sup>22</sup>

*Significance of Renal Lesions.*—Most observers have believed acute parenchymatous degeneration as it occurs in the convoluted tubules to be a mild type of cellular injury which is reversible.<sup>23</sup> Others<sup>24</sup> have maintained that it constitutes a morphologic expression of increased function or of stimulation. There seems to be little question, however, concerning the significance of hydropic, fatty and vacuolar degeneration of the tubular cells. These changes are accepted to indicate a more or less severe form of cellular injury.

Hyaline droplets have been noted in the renal epithelium in a wide variety of conditions. Terbrüggen<sup>25</sup> found an incidence of 66 per cent

TABLE 2.—Relationship of Jaundice to Albuminuria

	Cases	Cases with Given Degree of Albuminuria			
		0	Trace to +	++ to ++++	
Cirrhosis with jaundice.....	9	3	2		4
Cirrhosis without jaundice.....	11	5	3		3

in autopsies. He was convinced that these droplets represented a type of alteration different from acute parenchymatous degeneration, a conviction with which our observations are in agreement. Smetana and Johnson<sup>26</sup> concluded from their studies on the salamander that hyaline droplets represent protein stored in the tubular epithelium and that the protein is derived from the tubular urine. In our cases we have not been able to determine what the exact significance of hyaline droplets is, but we believe that it is not directly related to other degenerative lesions.

The relationship of the degenerative tubular lesions to the albuminuria is a matter of considerable interest. The incidence and the severity of albuminuria could not be definitely correlated with the presence or the absence of jaundice, although there is some suggestion in table 2 that

22. Moon, V.: Arch. Path. 18:381, 1934.

23. Karsner, H. T.: Human Pathology, Philadelphia, J. B. Lippincott Company, 1942.

24. Davidman, A., and Dolley, D. H.: J. M. Research 42:515, 1921.

25. Terbrüggen, A.: Beitr. z. path. Anat. u. z. allg. Path. 86:236, 1931.

26. Smetana, H., and Johnson, F. R.: Am. J. Path. 18:1029, 1942.

the albuminuria is intensified in the presence of jaundice. As Ratnoff and Patek<sup>27</sup> indicated, there is some difference of opinion concerning the incidence of albuminuria in cases of portal cirrhosis, but that it does occur, unexplained by other causes, is certain. Ratnoff and Patek stated that "apparently albuminuria occurs but there is no evidence that this is the result of anything more than congestion of the kidney resulting from the pressure of ascitic fluid on the renal vein." In our cases we find no evidence of marked hyperemia of the kidneys, gross or microscopic. We are inclined, therefore, to interpret the albuminuria as being due to the accompanying parenchymal injury of the kidney rather than to passive hyperemia. The albuminuria might be explained by a possible increase of permeability of the glomerular membrane or by an impairment of tubular reabsorption of the protein of the glomerular filtrate according to the concept of Dock.<sup>28</sup>

We are not able to say whether the oliguria and the azotemia seen frequently in this series of cases were due to the renal lesions or were prerenal in origin. Neither do we know what the end stage of the renal lesion is. There is no evidence that it progresses to a stage comparable to chronic glomerular nephritis or leads to clinical hypertension, but this is an interesting subject for speculation. It also seems possible that the renal lesions might predominate in some cases, giving a picture of renal disturbance with little or no evidence of hepatic disease.

#### SUMMARY

Pathologic changes occurring simultaneously in the liver and the kidneys under clinical conditions, and similar lesions produced experimentally in animals by means of diets deficient in lipotropic factors, have been described in the literature.

Twenty-five cases of relatively uncomplicated portal cirrhosis were studied by us to determine whether renal lesions were consistently present.

Degenerative changes of the convoluted tubules and to a lesser extent of the loops of Henle, consisting of granular swelling with or without hydropic, vacuolar and fatty degeneration and hyaline droplet formation, were encountered to some degree in all cases. In some cases evidence of a more chronic form of renal injury was revealed, characterized by dilatation, atrophy and regeneration of the convoluted tubules. Glomerular changes consisting of periglomerular fibrosis and hyaline thickening of the basement membrane were observed, but these could not be definitely attributed to the cirrhosis. Albuminous precipitate was frequently observed in the glomerular spaces.

27. Ratnoff, O. O., and Patek, A. J.: Medicine 21:207, 1942.

28. Dock, W.: New England J. Med. 227:633, 1942.

Albuminuria was present in 60 per cent of the cases, and this was attributed to renal parenchymal injury resulting in either an increase of glomerular permeability or a decrease of tubular function.

A direct correlation was demonstrated between the activity of the cirrhotic process and the severity of the renal lesions.

All degrees of tubular degeneration were observed in the cases with no jaundice as well as in those with jaundice, and the correlation between the activity of the cirrhosis and the degree of tubular injury was equally good in the two groups, although in general the severity of the tubular degeneration was somewhat greater in the group with jaundice.

Although it cannot at present be said with certainty, we believe that the renal changes described in these cases and in certain other types of cases in which hepatic and renal lesions occur simultaneously might result not directly or indirectly from injury of the liver but rather from the same factors which produce the injury of the liver.

## TRANSITORY PULMONARY INFILTRATIONS (LOEFFLER'S SYNDROME) IN RABBITS

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IN 1932 and again in 1936 Löffler reported on a transitory pulmonary disease characterized by a dearth<sup>1</sup> of clinical manifestations, by eosinophilia and by characteristic pneumonic infiltrations roentgenographically. Clinically, some of his patients presented general malaise and an irritating cough productive of a slight amount of yellowish mucoid sputum which was poor in cells and occasionally contained a few eosinophils. Physical examination was often disappointing but sometimes disclosed scattered rales and a fine friction rub. The leukocytes were increased to as much as 15,000 per cubic millimeter of blood and the sedimentation rate to 15 mm. per hour. In about one third of the cases there were no signs or symptoms, the disease being discovered by routine roentgenograms. These revealed unilateral or bilateral, single or multiple, homogeneous or spotty, sharp or ill defined shadows which appeared and disappeared in from three to eight days. Recovery ensued in all his cases, and for this reason the nature of the shadows was not determined. Löffler did not ascertain the cause of the disease in his 51 cases but believed that the lesion was a specific tissue reaction probably produced by a variety of antigens and comparable to erythema nodosa of the skin.

Although numerous case reports and several reviews have appeared in the literature since Löffler's publications, few<sup>2</sup> have added appreciably to his classic description. While most authors agree that the disease is an allergic response on the part of the lungs, the views regarding the causative allergens vary. Probably the most frequently mentioned are animal parasites, including such organisms as *Ascaris lumbricoides*,<sup>3</sup>

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1. Löffler, W.: Beitr. z. Klin. d. Tuberk. **79**:368, 1932; Schweiz. med. Wchnschr. **66**:1069, 1936.

2. (a) Freund, R., and Samuelson, S.: Arch. Int. Med. **66**:1215, 1940. (b) Peabody, J. W.: Dis. of Chest **10**:391, 1944.

3. (a) Müller, R. W.: Deutsche med. Wchnschr. **64**:1286, 1938. (b) Barker, C. S.: Canad. M. A. J. **40**:494, 1939. (c) Beck, L. C.: Hawaii M. J.

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Strongyloides stercoralis,<sup>4</sup> Fasciola hepatica and amebas.<sup>5</sup> Mycobacterium tuberculosis has been considered to be the etiologic agent by some,<sup>6</sup> but this has been denied by others.<sup>7</sup> More recently, many authors have stressed the association of the syndrome with bronchial asthma<sup>8</sup> and so have not only definitely established an allergic basis for the disease but indicated one of the common routes of invasion of the allergen, namely, the respiratory tract. In this connection two specific reports appear to be pertinent. The first was that of Engel,<sup>7b</sup> who stated that in China every year in May and June, when the privet flower blooms, there occurs a cough with expectoration of canary yellow sputum known as the "privet cough." The author himself had the cough on several occasions, at which times studies revealed the typical syndrome outlined by Löffler. Although the privet plant is said to produce little pollen in the air, the seasonal occurrence of the symptoms strongly suggests sensitivity to this allergen. A second pertinent report was that of Dickson,<sup>9</sup> who described the syndrome as observed in 2 laboratory workers suffering from coccidioidomycosis. Dust from dishes on which this fungus was growing was seen to permeate the air when the covers were removed, and it was the author's opinion that the infection was contracted by inhalation of this material.

Because patients with this disease do not as a rule die, most of the knowledge regarding its pathologic character has been speculative. Löffler<sup>1</sup> merely stated that it was a specific tissue reaction. Engel<sup>7b</sup> and later Baer<sup>10</sup> considered the condition as edema of the lungs, Soderling<sup>8a</sup> expressed the belief that the shadows were due to stagnation of secretion which combined with bronchiolar spasm to produce collapse of the lung, whereas Kartagener<sup>11</sup> and Maier<sup>7a</sup> regarded the lesion as true eosinophilic pneumonia. In considering more tangible evidence

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1:361, 1942. (d) Meyenberg, H.: Schweiz. med. Wchnschr. 72:809, 1942; abstracted, J. A. M. A. 121:626, 1943.

4. Berk, J. E.: J. A. M. A. 127:354, 1945.

5. Hoff, A., and Hicks, H. M.: Am. Rev. Tuberc. 45:194, 1942.

6. Leitner, J.: Beitr. z. Klin. d. Tuber. 88:388, 1936. Karan, A. A., and Singer, E.: Ann. Int. Med. 17:106, 1942. Peabody. <sup>2b</sup>

7. (a) Maier, C.: Helvet. med. acta 10:95, 1943; abstracted, J. A. M. A. 123:869, 1943. (b) Engel, D.: Beitr. z. Klin. d. Tuber. 87:239, 1935.

8. (a) Soderling, B.: Arch. Dis. Childhood 14:22, 1939. (b) Harkavy, J.: Arch. Int. Med. 67:709, 1941; (c) J. Allergy 14:507, 1942. (d) Hennell, H., and Sussman, M. L.: Radiology 44:328, 1945. Maier.<sup>7a</sup>

9. Dickson, E. C.: J. A. M. A. 111:1362, 1938.

10. Baer, A.: Ohio State M. J. 37:960, 1941.

11. Kartagener, M.: Schweiz. med. Wchnschr. 72:862, 1942; abstracted, J. A. M. A. 121:893, 1943.

of the pathologic process, the report by Smith and Alexander<sup>12</sup> of a case of the syndrome coming to necropsy must be questioned. Although their patient may have had the disease several months before death a subsequent leukocyte count of 45,600 per cubic millimeter of blood with 97 per cent lymphocytes strongly suggests lymphatic leukemia. Meyenberg's<sup>13</sup> publication is also open to question because he did not furnish proof of the transient nature in any of his 4 cases. In 1 case he described two small pulmonary foci that contained many eosinophils. In 2 cases there were bronchitis, bronchiolitis and bronchopneumonia, and in 1 case, lobar pneumonia. The paper by Harkavy<sup>14</sup> on 15 cases of bronchial asthma is the first to furnish acceptable data on the pathologic nature of the pulmonic lesions. At least 3 of the 4 cases that came to autopsy might be considered as typical instances of Löffler's syndrome. In them he noted (1) eosinophilic infiltration, edema and hyalinization of the basement membrane of the bronchial mucosa, (2) infiltration of the alveolar septums with eosinophils, polymorphonuclear leukocytes and lymphocytes, (3) thrombosis of the small vessels and (4) congestion, edema, atelectasis and emphysema of the avleoli. In another case of the syndrome associated with bronchial asthma that came to necropsy Broch<sup>15</sup> also described pulmonary congestion, edema and diffuse infiltration of the alveoli, septums and bronchi with a variety of cells, among which eosinophils were particularly conspicuous.

Experimentally, several authors have produced allergic pneumonia in animals. The earlier literature on attempts to produce asthma in guinea pigs has been summarized by Alexander, Becke and Holmes.<sup>16</sup> These authors sensitized guinea pigs to horse dander and egg white and then by sprayings of each of these allergens and direct intratracheal injections of the egg white they were able to produce typical asthmatic attacks in many animals with immediate death in some of them. They did not examine the lungs microscopically. Fried<sup>17</sup> sensitized rabbits to horse serum, after which he injected a small amount of the serum intratracheally and killed the animals at intervals of from one hour to several weeks. Microscopically there were within a few hours edema, granulocytic infiltration, atelectasis and periphlebitis. This was followed in twelve to twenty-four hours by consolidation, in which mononuclear cells and mononuclear eosinophils composed the bulk of the intra-alveolar exudate, and in forty-eight hours by definite lobar consolidation, accompanied by exudate in the pleural cavities. In four days the alveolar exudate began to organize and disappear. Some vessels showed

12. Smith, D. C. W., and Alexander, A. J.: *South. M. J.* **32**:267, 1939.

13. Broch, O. J.: *Acta med. Scandinav.* **118**:311, 1943.

14. Alexander, H. L.; Becke, W. G., and Holmes, J. A.: *J. Immunol.* **11**:175, 1926.

15. Fried, R. M.: *J. Exper. Med.* **57**:111, 1933.

a cuffing of lymphocytes, swelling and proliferation of the intima, and thrombosis. Cannon, Walsh and Marshall<sup>16</sup> reported an acute anaphylactic inflammation of the lungs of rabbits sensitized to egg white and crystalline egg albumin. The lesions consisted of edema, alveolitis, bronchitis, pneumonic consolidation and acute arteritis and phlebitis. Despite these reports, however, and despite the ever increasing literature on Löffler's syndrome in man, no one has attempted to follow the allergic pneumonia in animals roentgenographically and to correlate these observations with the accompanying clinical manifestations and pathologic alterations. In other words, no one has attempted to reproduce in animals the syndrome as reported by Löffler and others in man. For this reason, and to understand better the underlying pathologic changes in man which are so meagerly reported, the following experiment was undertaken.

#### MATERIAL AND METHOD

After several preliminary trials 13 rabbits, each weighing approximately 2,200 Gm., were sensitized to horse serum by subcutaneous abdominal injections of 0.5 cc., made at the rate of one every other day until a total of nine injections had been given. Eighteen days after the last injection 0.1 cc. of serum was introduced into the skin of the lateral portion of the abdomen several inches away from the site of the sensitizing injections. Two days later the hair over the ventral surface of the neck was clipped, and under light ether anesthesia the trachea was exposed. This procedure was also carried out on 9 normal rabbits. The rabbits were then divided into two groups. Group 1 consisted of 6 sensitized rabbits (5, 6, 7, 8, 9 and 11) and 4 controls (17, 18, 19 and 20). Each of these received a single intratracheal instillation of 5 cc. of horse serum. Group 2 consisted of 7 sensitized animals (1, 2, 4, 10, 12, 94 and 95) and 5 controls (13, 14, 15, 16 and 66). Each of these received a preliminary intratracheal instillation of 0.5 cc. of horse serum and subsequent instillations of a similar amount at hourly intervals until a total of from 5 to 12 doses had been received (five on the first day and the remainder on the second day). Three rabbits (94, 95 and 66) received an additional intratracheal instillation of 1 cc. on the fourth day. The cervical wound in each animal was closed when the allotted instillations were completed. All rabbits received the initial instillations while they were still partially anesthetized. The rectal temperature, a differential blood smear and a roentgenogram of the thorax had been recorded before the trachea was exposed. Roentgenograms were taken again twenty-four hours after the single instillation in group 1 and three hours after the multiple instillations had been completed on the second day in group 2. Subsequently roentgen examinations were made at intervals of one and several days depending on the progress of the pneumonia, and of all animals at the time of death. Blood smears were made and rectal temperatures were recorded to accompany the roentgenograms until it was certain that they were stabilized. All roentgenograms were made with the rabbits in an erect position, stretched out on a specially constructed board, and a small focal spot rotating anode tube was used, with 300 milliamperes of current and 70 kilovolts at 1/120 second and a distance of 36 inches (91.5 cm.). The animals died or were killed (by an

16. Cannon, P. R.; Walsh, T. E., and Marshall, C. E.: Am. J. Path. 17:777, 1941.

intravenous injection of ether) at intervals of from twelve hours to fifteen days and the trachea and the lungs were examined grossly and microscopically. While still *in situ* the trachea was carefully opened and a smear made of its secretions. This was stained for eosinophils by applying first Wright's stain in the usual manner, decolorizing in 70 per cent alcohol and counterstaining with methylene blue. At least 300 leukocytes were counted, and only those with unmistakably large eosinophilic granules were considered as eosinophils. In addition to the tracheas and lungs of the aforementioned 22 animals we studied microscopically those of several normal untreated rabbits and those of others treated exactly like the rabbits in group 2 except that the lesions were not followed roentgenographically.

## RESULTS

*Clinical Manifestations.*—All sensitized animals reacted positively to the intracutaneous injection of horse serum. Within five hours after the injection an area of erythema and edema appeared that measured as much as 5 cm. across and 1.2 cm. in depth. In twenty-four hours the area of erythema increased to as much as 7 cm. in diameter, with a central dark region of beginning necrosis appearing in it in some of the rabbits. The reaction reached its maximum intensity in forty-eight hours, after which the erythema and the edema subsided and the necrosis sloughed. The control rabbits in group 1 showed no untoward signs following the intratracheal instillation of the serum. In all the sensitized animals in this group a few rales developed, which cleared in several hours, but only in 1 did anaphylaxis develop. About two minutes after receiving an injection rabbit 5 began to cough, sneeze, shake his head, thresh around and breathe rapidly, so that for about thirty seconds it appeared as though he would die. He promptly recovered, however, and remained visibly well thereafter. There were no severe reactions in the sensitized rabbits of group 2. Some of the animals, particularly 10, 2 and 1, showed evidence of obstruction subsequent to the second and third instillations. Inspiratory dyspnea was unmistakable and became increasingly severe with each instillation. Rabbit 10 was found dead in the cage five hours after the last instillation on the first day and rabbits 2 and 1 became extremely cyanotic and dyspneic after the first and the second instillation, respectively, on the second day. Each ceased to breathe a few minutes later. In both these and the remaining sensitized rabbits rales and snorting developed after the initial intratracheal instillations. The former disappeared shortly after the instillations were stopped, but the latter persisted for several days. Both rales and snorting were also present in 2 of the control animals of group 2 but were not nearly as apparent as they were in the sensitized rabbits, nor did they last as long. None of the controls showed cyanosis or inspiratory dyspnea.

There was no significant alteration of the temperature of any of the animals. The highest recorded rise was 2 F., but in some there was a drop of 2 F. below the previous normal, and the changes were similar in all the animals. Changes in the numbers of eosinophils in the blood smears were likewise insignificant. Three sensitized rabbits (5, 7 and 9), whose normal eosinophils constituted 3, 8 and 0 per cent of the total leukocyte count, showed an increase to 11, 23 and 16 per cent, respectively, but these were counterbalanced by an increase in control animals (14 and 16) from 2 to 31 per cent and 1 to 11 per cent respectively. The remaining animals in both groups showed no noteworthy changes.

*Roentgenographic Changes.*—Serial roentgenograms showed the development of transitory pulmonary infiltrations that reached their maximum intensity in from twenty-four to seventy-two hours after intratracheal instillation and then cleared

rapidly in from seven to thirteen days. The distribution of the pulmonary lesions was by no means uniform. One or more lung fields were involved in single or multiple areas. The extent of the consolidation varied considerably; in some cases there was complete lobar distribution, in others dense areas radiated outward from the hilar regions, and in still others there were small, fairly discrete nodular infiltrations of the lung parenchyma. The shadows were usually dense and fairly well demarcated, although sometimes they were ill defined. None of the controls had isolated lesions peripherally in the lung fields, nor was involvement of the pleura noted (figs. 1 and 2),

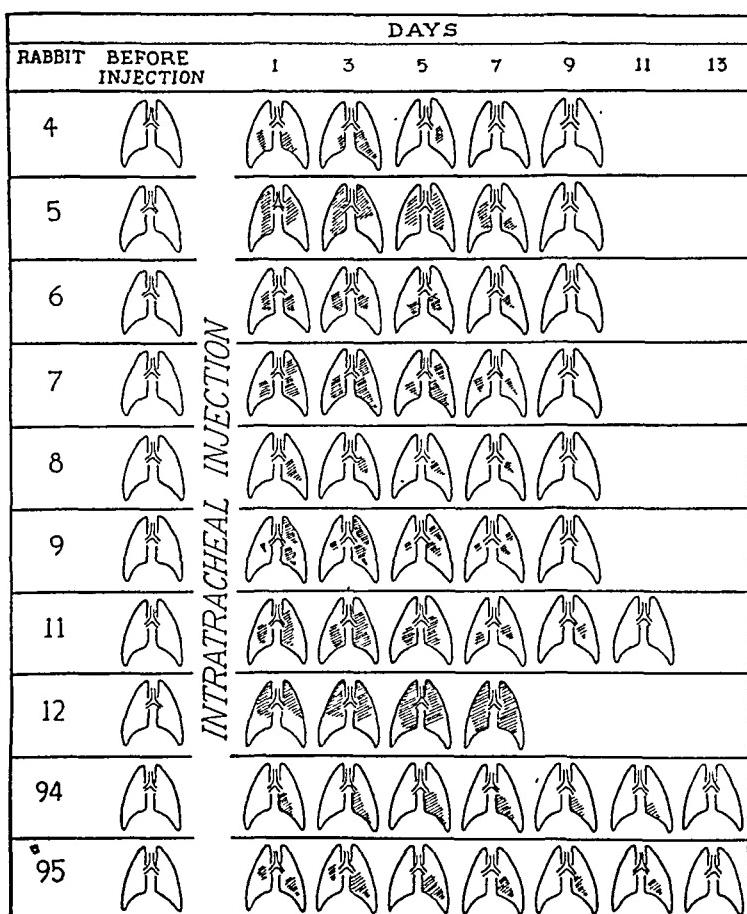


Fig. 1.—Distribution and duration of pulmonic infiltrations shown by serial roentgenograms.

More specifically, in group 1, with single instillations being made, all the sensitized rabbits (5, 6, 7, 8, 9 and 11) showed pulmonic infiltrations, which were variable in degree and distribution, as can be seen from figure 1. These infiltrations reached a maximum degree in from twenty-four (8) to forty-eight (5, 6, 7, 9 and 11) hours and showed complete clearing roentgenographically in from nine (5, 6, 7, 8 and 9) to eleven (11) days. None of the controls in this group had demonstrable pulmonary lesions. In group 2, with multiple instillations being given, all sensitized rabbits (1, 2, 4, 12, 94 and 95) had significant pulmonary infiltrations within twenty-four hours after the first intratracheal instillation. Rabbits 1 and 2 showed extensive consolidation of both upper lobes, together

with considerable involvement of the inner zones of the lower lobes in rabbit 1 and more extensive infiltration of both lower lobes in rabbit 2. These animals died twenty-four hours after the first instillation. In rabbit 12 the pulmonic process involved both upper lobes, the middle lobe of the right lung and the inner zone of the lower lobe of the left lung—all within twenty-four hours. Instead of clearing, the consolidation became progressively more dense. In forty-eight hours there was atelectasis of the middle lobe of the right lung, followed later by bilateral empyema. The animal died on the eighth day. The lesions in rabbit 4 cleared in seven days, and those in rabbits 94 and 95 cleared in thirteen days.

*Pathologic Changes.*—In all the sensitized rabbits of group 1 pneumonia developed that cleared roentgenographically, and at autopsy the lungs of all rabbits



Fig. 2.—Roentgenograms of rabbits 5 and 94 taken before intratracheal instillation of serum, at the height of the pneumonic process and after clearing.

but 5 and 11 were entirely normal. The former showed a group of small abscesses of a total diameter of 6 mm. in the medial portion of the lower lobe of the left lung. The latter had 2 areas of atelectasis, each 3 mm. in diameter; one was at the tip of the lower lobe of the left lung, and the other was at the base of the upper lobe of the right lung. The roentgenograms of the controls in this group did not present shadows at any time, and at autopsy the lungs of these rabbits were normal. The peritracheal tissue was free of infection in each rabbit of this group. In group 2, 3 of the 7 sensitized rabbits (10, 2 and 1) died on the first and second days of the instillation of serum. In a fourth rabbit (12) a peritracheal abscess developed, which extended into the mediastinum and each pleural cavity, producing bilateral empyema. In the remaining 3 animals (4, 94 and 95) pneumonia

developed that cleared roentgenographically, but at postmortem examination the lungs of rabbits 4 and 95 only were clear, whereas the medial portion of the lower lobe of the left lung of rabbit 94 contained a focus of several tiny conglomerate abscesses that measured 9 by 4 mm. The roentgenograms of 3 of the controls (66, 14 and 15) in group 2 did not present shadows, but those of 2 (13 and 16) showed pulmonic infiltrations that first appeared at twenty-four hours. Each of the latter at autopsy showed a peritracheal and mediastinal abscess and bilateral empyema. Rabbit 13 disclosed in addition massive atelectasis of each upper lobe. In all animals of group 2 except 4, 94, 95, 66 and 14 a peritracheal abscess developed, and, as already stated, in rabbits 12, 13 and 16 it trekked down the mediastinum and was continuous with pleural empyema.

The lungs as examined at various intervals of time after intratracheal instillation of the serum was completed disclosed the following pathologic changes: Within twelve hours there was intense congestion of the mucosa of the larynx, the trachea and the bronchi, with no obstruction of the lumen. The lungs showed petechiae and atelectasis. Sometimes the latter was massive and occupied an entire lobe, but usually it was focal and irregularly distributed. Microscopic sections of the trachea disclosed some sloughing of the epithelial cells, intense engorgement of the submucosal capillaries and only a sprinkling of plasma cells, lymphocytes, eosinophils and polymorphonuclear leukocytes. Sections of the lungs showed extreme congestion, with edematous fluid filling most of the alveolar spaces and disrupting the alveolar walls (fig. 3 A). Inflammatory cells were entirely absent. Peripherally there were extensive areas of emphysema wherein the alveoli were greatly distended and their septums often irregularly ruptured. The lymphatic vessels were inconspicuous, and the arteries were normal.

At twenty-four hours the lumen of the tracheobronchial tree was still patent but filled with frothy serosanguineous fluid, and the mucosa was congested. Besides atelectasis, congestion and petechiae, the lungs disclosed irregularly shaped and distributed areas of pneumonia. The portions not involved by the aforementioned processes were voluminous (at least twice the normal size) and comparatively pale. Histologic sections of the trachea and the larger bronchi showed in addition to the previously mentioned alterations vacuolation of the superficial epithelial cells, fibrinous degeneration of the connective tissue beneath the basement membrane and diffuse infiltration with eosinophils and a few plasma cells (fig. 3 B). Thrombi were present in some of the submucosal vessels. The epithelium of the bronchioles was essentially the same, but in some areas it was disrupted by an intense eosinophilic infiltration. Many of the lumens were completely plugged with eosinophils or mixtures of these cells, sloughed epithelial cells and debris. Sections of the lungs revealed some areas in which only atelectasis, acute emphysema or pneumonia was present and other areas in which there was intermingling of these three processes (fig. 3 C). The consolidated areas were either large and diffuse or small and patchy and showed some congestion and edema but more conspicuously infiltration with eosinophils, which in some areas composed 100 per cent of the infiltrating cells. In other sections they were less conspicuous but nevertheless always present. The remaining cells were chiefly polymorphonuclear leukocytes and plasma cells. The perivascular lymphatics were distended with edematous fluid, and some arteries showed swelling and vacuolation of the endothelial cells. There was no pleuritis.

At forty-eight hours the only gross differences of the lungs as compared with those already described were that the visceral pleura was covered with a thin fibrinopurulent exudate and consolidation was more diffuse, so that sometimes an entire lobe was involved. Histologically there were still present areas of congestion,

edema, atelectasis and emphysema. In some sections the pneumonia was diffuse and of the lobar type with a uniform infiltration that was almost 100 per cent eosinophils (fig. 3 C). In other areas, however, the exudate was less dense and for the first time disclosed large round vacuolated phagocytes. The pleural exudate consisted of many neutrophils and fewer eosinophils intermingled with varied amounts of fibrin. Some of the arteries were cuffed with eosinophils, but none showed lesions resembling periarteritis nodosa. The perivascular lymphatics were distended with serum and conspicuous.

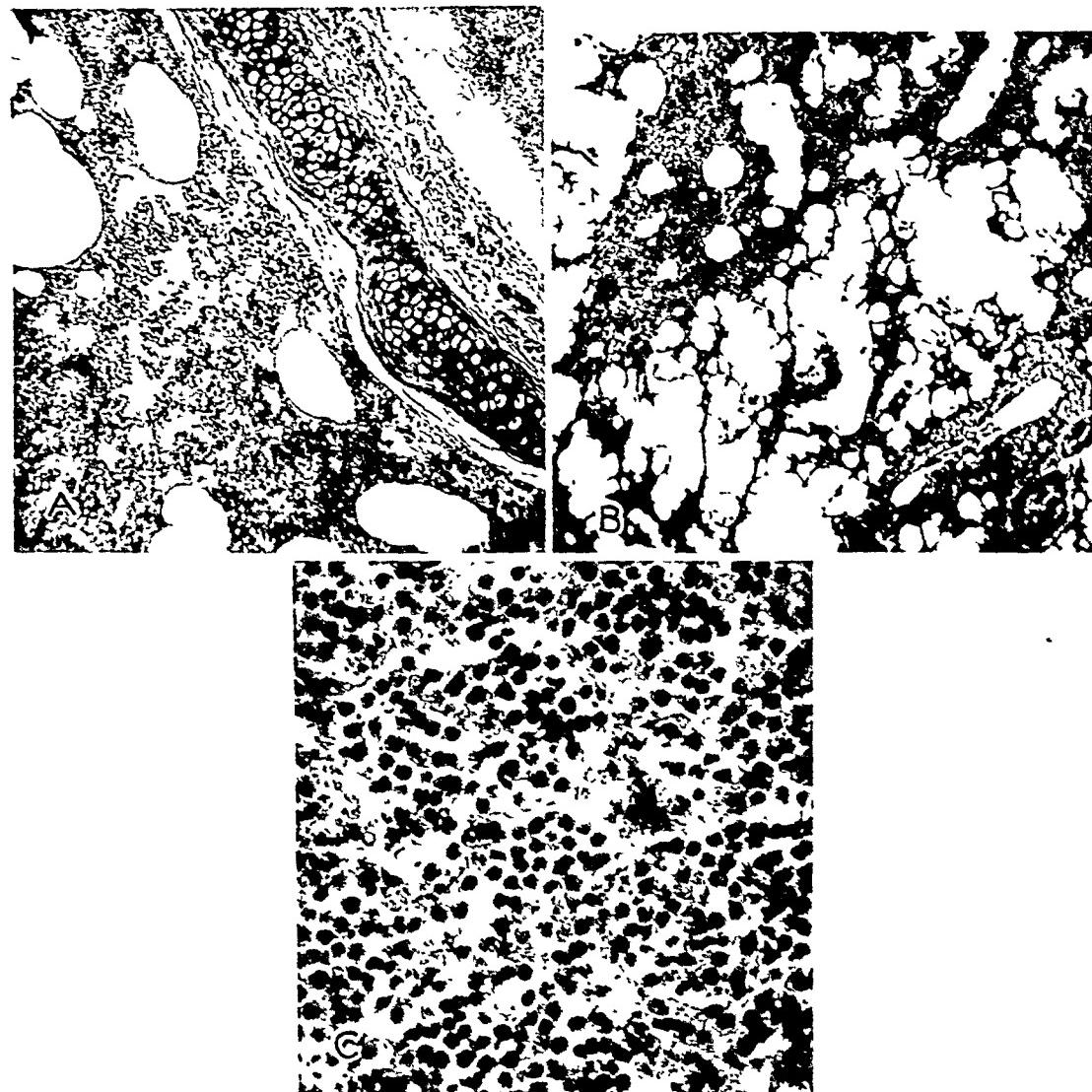


Fig. 3.—A, section of a bronchus and adjoining lung made twelve hours after intratracheal instillation of serum was completed, showing congestion of the alveoli and edematous fluid in the alveolar spaces and the bronchial lumen. Hematoxylin and eosin;  $\times 75$ .

B, section of a lung made twenty-four hours after instillation of serum was completed, showing areas of atelectasis and pneumonia alternating with areas of acute emphysema. Hematoxylin and eosin;  $\times 37.5$ .

C, section of a diffusely consolidated lung made forty-eight hours after instillation of serum was completed, showing faint alveoli in the background and a dense infiltrate of eosinophils. Hematoxylin and eosin;  $\times 400$ .

The lungs examined at seventy-two hours were essentially the same in gross appearance as those examined at forty-eight hours. Microscopic sections of the trachea showed normal mucosa and submucosa. In some areas the lungs were similar microscopically to those examined at forty-eight hours, but in others they revealed the following differences: The septums were thick, prominent and cellular; the exudate was heavier, contained more fibrin and was well on the way to organization, and large intra-alveolar phagocytes were particularly conspicuous. Because we did not have as many animals as we wished, none was killed after seventy-two hours until complications arose or until roentgenograms showed normal lung shadows. We assumed, therefore, that there was a continuation of organization and resolution along the lines seen at the seventy-two hour interval in the rabbits in which a pneumonic process developed that cleared, with the lungs appearing normal at autopsy.

Three significant complications developed in a few rabbits, namely, (1) residual focal pulmonary abscesses in 3 sensitized animals (12, 94 and 5), (2) empyema in 1 sensitized rabbit (12) and 2 controls (13 and 16) and (3) peritracheal abscesses in one half of the rabbits receiving multiple instillations. Histologic sections of the earliest abscesses disclosed central areas of debris and nuclear fragments, sharply demarcated from the immediately surrounding collapsed alveoli, which contained phagocytes, and beginning proliferation of fibrous tissue. The older abscesses showed calcific deposits in the central areas, around which were a few spurious giant cells. Peripheral to this were nuclear fragments intermingled with few neutrophils and phagocytes. Beyond these there was a zone of granulation tissue consisting of a background of young fibroblasts rich in capillaries and diffusely infiltrated by eosinophils and scattered neutrophils, lymphocytes, plasma cells and phagocytes. These pulmonic abscesses were in every microscopic detail duplicates of the cutaneous abscesses formed at the sites of intradermal inoculation and of the peritracheal abscesses formed in the sensitized rabbits. They differed from those formed in the necks of unsensitized rabbits in that the latter contained few or no eosinophils. The septums about some of the abscesses and particularly those beneath the pleura showed some thickening and fibrosis and were infiltrated with plasma cells and lymphocytes. Some were lined with tall cuboidal cells identical with those lining the bronchioles, and their lumens contained many vacuolated phagocytes mixed with a few plasma cells and lymphocytes. Histologic sections of the pleura in each of the 3 rabbits in which empyema developed disclosed a mixture of neutrophils, plasma cells and other monocytes, granular debris and some fibrin. All were devoid of eosinophils.

Eleven of the 22 rabbits showed eosinophils in the tracheal secretion. Of these, 3 were control rabbits 13, 66 and 15, disclosing 1, 2 and 5 per cent eosinophils, respectively, whereas the remaining 8 were sensitized rabbits, 95, 10, 7, 6, 1, 2, 9 and 5, which disclosed, respectively, 7, 12, 20, 21, 30, 64, 67 and 81 eosinophils.

#### COMMENT

Although it is difficult to evaluate clinical manifestations in the rabbit and particularly to correlate them with those in man, several more obvious observations do nevertheless appear to be in order. All authors have repeatedly stressed scarcity of signs and symptoms of the condition known as Löffler's syndrome as it develops in man. This dearth of clinical manifestations has also been noted in the rabbits in which the

lesions were produced experimentally. Anaphylaxis following the first intratracheal instillation of serum was probably avoided in all our animals except 1 by giving the instillation while the anesthesia was still effective. In rabbit 5 coughing, sneezing, threshing about and rapid breathing were observed a few minutes after the animal received the serum, but in the remaining sensitized animals in group 1 only a few rales developed, which cleared in several hours. In group 2 (which received multiple instillations) inspiratory dyspnea became manifest in several of the sensitized animals and snorting in most of them. The former was obviously not a sign of bronchial asthma, because in this condition the dyspnea is expiratory. At autopsy none of the rabbits showed obstruction of the larynx or the trachea, but the lumen of the tracheobronchial tree was filled with edematous fluid and the smaller bronchi and bronchioles were plugged with desquamated epithelium and cells of inflammatory origin. These changes appeared to be sufficient to account for the dyspnea. There was no evidence of spasm of the bronchi or the bronchioles. Snorting, unless it was a manifestation of dyspnea, could not be accounted for. The rales that appeared in the sensitized animals of group 2 were only transitory and disappeared shortly after the instillations were stopped. In all instances they were probably caused by the instilled serum plus the edematous fluid, which microscopically was conspicuous.

Another similarity between the syndrome occurring in man and that produced in rabbits is the lack of disturbance of the temperature. In man, if fever is present, it is slight. In none of the animals did the reading differ more than 2 F. from the previous normal, and the temperature changes in the control rabbits were similar to those in the sensitized. Although the eosinophilic count of the sputum of man has been infrequently recorded, there are indications that it is often increased.<sup>17</sup> There was an unequivocal increase in that of rabbits. Of the 11 animals that showed eosinophils in smears of tracheal secretions, only 3 were controls, disclosing counts of 1 to 5 per cent, whereas 8 were sensitized rabbits, which revealed counts of 7 to 81 per cent. The same parallelism, however, did not hold regarding the eosinophils of the blood stream. In listing the criteria for a diagnosis of the syndrome in man almost all authors include eosinophilia. Soderling,<sup>8a</sup> however, stated that too much significance should not be attached to an increase of eosinophils in the blood, for such cells may be temporarily or entirely absent. Cases without eosinophilia, albeit in the minority, have been reported.<sup>18</sup> The rabbits' blood smears showed no appreciable alteration

17. Löffler.<sup>1</sup> Harkavy.<sup>8b</sup> Kartagener.<sup>11</sup>

18. Beck.<sup>3c</sup> Peabody.<sup>2b</sup>

of the eosinophilic count. Thus, in summing up the clinical aspects one may say that the constitutional disturbances in the syndrome as produced in rabbits were minimal, and so paralleled closely those seen in man.

There was also a close correlation between the roentgenograms of man and those of the rabbits in which the lesions were produced experimentally. Löffler<sup>1</sup> described the former as showing homogeneous or, more often, spotty or cloudlike, sharply or less sharply limited, single or multiple, unilateral or bilateral, migratory shadows that appeared and disappeared in from three to eight days. Occasionally they also showed localized areas of pleural involvement and small pockets of loculated fluid. Depending on the distribution of the shadows, he divided his cases into the following five groups: (1) those in which the roentgenograms presented large, more or less irregular unilateral or bilateral shadows, (2) those in which the shadows represented small round foci, (3) those in which multiple lesions were shown in one or both lungs, (4) those in which the lesions were sharply limited to a lobe and (5) those in which the lesions resembled tuberculous infiltrations. In the roentgenograms of the rabbits the shadows were for the most part confluent and spreading outward from the hilus. Some, however, were spotty and nodular and were seen in the inner zones of the lung fields. There were no rounded foci at the periphery, nor were there visible involvements of the pleura or collections of fluid except in those few animals in which empyema developed. The chief difference between the shadows of man and those of rabbits was the lack of migration of the latter. In animals the areas of infiltration developed to a maximum and then cleared without reappearing or shifting to other portions of the lung fields. The reason for this difference may lie in the fact that in man the allergen is present over a prolonged period, whereas in all but 2 experimental animals the lungs were not exposed to the allergen after the initial instillation or series of instillations was completed.

The pathologic changes observed in the lungs of rabbits followed closely those seen in man in the few recorded instances in which the human patient died of the syndrome.<sup>19</sup> In the latter the changes consisted of: hyalinization of the basement membrane of the bronchial epithelium, together with edema and eosinophilic infiltration of the submucosa; infiltration of the alveolar walls with eosinophils, neutrophils and lymphocytes; thrombosis of small vessels, and, in the alveoli, congestion, edema, atelectasis and emphysema. In rabbits the only difference in the walls of the trachea and the bronchi was absence of hyalinization of

19. Harkavy,<sup>8c</sup> Broch.<sup>13</sup>

the basement membrane: In the lung proper the changes consisted of congestion, edema, atelectasis, emphysema and eosinophilic pneumonia.

As a rule, the lesions in man clear completely, leaving no residuum roentgenographically. This was also true in all animals of group 1 and in most of the surviving ones of group 2. Pathologically, however, 3 of these rabbits disclosed single groups of conglomerate abscesses. In 2 animals the abscesses were located in the medial portion of the lower lobe of the left lung and were obscured by the shadow of the heart, and in a third they were obscured by empyema. That these abscesses were the remains of allergic pneumonia is attested by their microscopic similarity to the lesions produced at the sites of intradermal injection. If the rabbits had lived longer, even these foci might have resolved completely or become fibrosed. One other animal (11) in which the lesions cleared roentgenographically disclosed at autopsy two tiny residual areas of atelectasis. There is, of course, no way of telling whether such complications do or do not sometimes follow the syndrome in man. The formation of peritracheal abscesses in group 2 was undoubtedly the result of direct external contamination in all animals together with a specific allergic response to extravasated serum in the sensitized ones. The latter view is borne out by the fact that eosinophils were present in the abscess walls of the sensitized rabbits and were not present in those of the controls. Because empyema was observed only in those rabbits that showed peritracheal and mediastinal abscesses it was considered as a direct extension of the infection of the neck and the mediastinum and not as a result of the pneumonitis. Empyema therefore has no part in the syndrome and in our experiment was an accidental complication in 3 rabbits.

#### SUMMARY

From a review of the literature it is apparent that most of the knowledge of the pathologic changes occurring in the lungs of man in Löffler's syndrome has been speculative. In two acceptable reports the lesions are listed as hyalinization of the basement membrane of the mucosa of the bronchi together with edema and eosinophilic infiltration of the submucosa, and congestion, edema, atelectasis, emphysema and eosinophilic pneumonia of the lung proper.

In the present experiment we have succeeded in duplicating in rabbits the syndrome as observed in man. Sensitized animals were given single or multiple intratracheal instillations of horse serum. They presented only slight clinical disturbances; roentgenographically they revealed transitory pulmonic infiltrations that cleared in from seven to thirteen days; there were eosinophils in the tracheal secretions, and pathologically there were congestion, edema and eosinophilic infiltration of the sub-

mucosa of the trachea and the bronchi, and in the lung parenchyma there were congestion, edema, atelectasis, emphysema and an eosinophilic pneumonia. The syndrome in rabbits differed from that usually seen in man in that (1) there was no eosinophilia and (2) the pulmonic shadows were not migratory.

It is concluded that Löffler's syndrome is an allergic inflammation of the lungs and that one route by which the allergen invades is that of inhalation.

# ADRENAL HEMORRHAGES IN MENINGOCOCCIC SEPSIS

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A FULMINANT type of sepsis, with purpura and cyanosis of the skin and adrenal hemorrhages, frequently occurring in the presence of a thymolymphatic constitution (especially in children) and generally caused by *Neisseria meningitidis* (*Neisseria intracellularis*), is wrongly given the name "Waterhouse-Friderichsen syndrome."

Neither Waterhouse<sup>1</sup> nor Friderichsen<sup>2</sup> was the first to describe this syndrome, but Voelcker,<sup>3</sup> in 1894. Graham-Little<sup>4</sup> compiled all previously published cases in 1901, and only after several other authors. Waterhouse<sup>1</sup> in 1911 and Friderichsen<sup>2</sup> in 1918 published studies, whose merit does not justify the use of the name "Waterhouse-Friderichsen syndrome" suggested by Thomas<sup>5</sup> and later by Glanzmann<sup>6</sup> and others.<sup>7</sup>

It seems that a descriptive term such as "fulminant sepsis with adrenal hemorrhage" (Schwarz<sup>8</sup>) or "the meningococcic adrenal syndrome" (Banks and McCartney<sup>9</sup>; Holmes and Cowan<sup>8</sup>) would be more appropriate and less erroneous than the present name.

The bacterium most frequently found in cases of the syndrome is *Neisseria meningitidis* and the presence of other bacteria described by some authors could not have been verified by modern methods. It seems

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1. Waterhouse, R.: *Lancet* **1**:577, 1911.
2. Friderichsen, C.: *Jahrb. f. Kinderh.* **87**:109, 1918.
3. Voelcker, A. F.: *Pathological Reports of the Middlesex Hospital*, London, 1894, p. 279.
4. Graham-Little, E.: *Brit. J. Dermat.* **13**:445, 1901.
5. Thomas, E., in von Pfaundler, M., and Schlossmann, A.: *The Diseases of Children*, translated by M. G. Peterman, Philadelphia, J. B. Lippincott Company, 1935, vol. 2.
6. Glanzmann, E.: *Jahrb. f. Kinderh.* **139**:49, 1933.
7. (a) Aegeerter, E. E.: *J. A. M. A.* **106**:1715, 1936. (b) Banks, H. S., and McCartney, J. E.: *Lancet* **1**:771, 1943. (c) Herbut, P. A., and Manges, W. E.: *Arch. Path.* **36**:413, 1943. (d) Lindsay, J. W., and others: *Am. J. M. Sc.* **201**:263, 1941. (e) Rabinowitz, M.: *ibid.* **166**:513, 1923. (f) Sacks, M. S.: *Ann. Int. Med.* **10**:1105, 1937. (g) Schwarz, J.: *Rev. sud-am. morfol.* **2**:69, 1944. (h) Thomas, H. B., and Leiphart, C. D.: *J. A. M. A.* **125**:884, 1944.
8. Holmes, J. M., and Cowan, J. M.: *Lancet* **1**:13, 1945.

that Andrewes,<sup>9</sup> in 1906, was the first to announce the meningococcus as the agent causing the syndrome, but this achievement is attributed by most authors to MacLagan and Cooke<sup>10</sup> in spite of a gap of ten years between both publications.

Descriptions of the intracranial anatomic lesions vary from "none" to presence of purulent meningitis or encephalitis (Banks and McCartney<sup>11</sup>), but in several cases in which macroscopically there were no lesions microscopic meningitis has been demonstrated (Schwarz<sup>7g</sup>); therefore, in cases in which clinically there are no meningeal signs and in which macroscopic inspection of the leptomeningeal appears to yield negative results there may be definitive inflammation of the latter.

A large thymus and hyperplastic lymph nodes and follicles were observed by numerous authors, notably by Baumann,<sup>12a</sup> Ghon,<sup>12b</sup> Mitchell and Rittershofer<sup>12c</sup> Aegeerter,<sup>7a</sup> Herbut and Manges,<sup>7e</sup> Rabino-witz,<sup>7e</sup> Schwarz and co-workers,<sup>13</sup> Martland<sup>14</sup> and recently by Holmes and Cowan,<sup>8</sup> who reported the case of an 8 year old girl with a thymus of the enormous weight of 41 Gm. The humoral chemism of the syndrome is not completely known yet, because of the swift development of the disease (six to forty-eight hours).

During the years 1941 to 1944 there was an epidemic of meningococcic meningitis in Chile. Not less than 9,669 cases were reported to the National Health Service; it may be supposed that the real number was much larger. Fulminant sepsis with adrenal hemorrhages was observed in more than 100 children, being especially prevalent during 1942 and 1943, and in practically all it came to a lethal end (Meneghelli<sup>15</sup>; Steeger<sup>16</sup>; Simpfendorfer<sup>17</sup>).

The present study concerns the histologic appearance of the adrenal glands in 5 infantile cases (table).

9. Andrewes, F. W.: Lancet **1**:1172, 1906.
10. MacLagan, P. W., and Cooke, W. E.: Lancet **2**:1054, 1916.
11. Banks, H. S., and McCartney, J. E.: Lancet **1**:219, 1942.
12. (a) Baumann, T.: Ztschr. f. Kinderh. **51**:276, 1931. (b) Ghon, A.: Med. Klin. **30**:695, 1934. (c) Mitchell, G., and Rittershofer, C. R.: The Thymus Gland, in Brennemann, J.: Practice of Pediatrics, Hagerstown, Md., W. F. Prior Company, Inc., 1944, vol. 3, chap. 22.
13. Meneghelli, J.; Schwarz, J., and Steeger, A.: Rev. chilena de pediat. **15**:1010, 1944. Schwarz.<sup>7g</sup>
14. Martland, H. S.: Arch. Path. **37**:147, 1944.
15. Meneghelli, J.: Rev. chilena de pediat., to be published. Meneghelli and others.<sup>13</sup>
16. Steeger, A., and Banfi, E.: Rev. méd. de Chile **71**:426, 1943.
17. Simpfendorfer, E.: Rev. chilena de pediat. **15**:1027, 1944.

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF THE  
ADRENAL GLANDS

**CASE 1.**—Both adrenal glands contained hemorrhagic zones. Microscopically, the glands, which were narrow had numerous partially coalescing hemorrhagic foci in the medulla and intense hyperemia of the cortex, with extreme dilatation of the capillaries, especially those in the two inner layers of the cortex. The capillary dilatation was intense enough to compress and flatten the cortical tissue. No difference was noted between the left and the right gland. No inflammatory lesions were seen.

**CASE 2.**—The left adrenal gland seemed normal; the right gland presented intense hyperemia in its lower two thirds.

Microscopically, the left gland showed intense hyperemia of the medulla with several small hemorrhages. The right gland showed intense hyperemia with

*Summary of Observations in Five Cases of Fulminant Meningococcic Sepsis*

Case; Sex; Age of Patient	Duration of Life After Onset of Illness, Hr.	Meninges		Weight of Thymus, Gm.	Weight of Brain, Gm.	Skin		Cerebro- spinal Fluid	Neisseria Menin- gitidis	Ker- nig's Sign
		Macro- scopic	Micro- scopic			Cya- nosis	Pur- pura			
1, M 10 yr.	20	Opal- escent	Menin- gitis	45	1,350	+++	+++	?	?	+
2, M 2 mo.	29	Puru- lent	Menin- gitis	35	?	+	+	Puru- lent	+	0
3, F 2 mo.	9	Clear	Menin- gitis	30	570	+++	+	?	+	0
4, F 1 yr.	15	Clear	Menin- gitis	38	1,120	+++	+++	Normal	0	0
5, F 3 mo.	15	Clear	Menin- gitis	15	660	+++	+++	Normal	0	0

circumscribed hemorrhages which were partially confluent in the medulla. The structure of the cortical zone was completely conserved. There was not even hyperemia. No inflammatory lesions were seen. The thickness of the glands was greatly reduced.

**CASE 3.**—The adrenal glands were completely destroyed by hemorrhages.

Microscopically, the lesions were almost the same in both glands. The thickness of the glands was much reduced. Both medullas were completely destroyed by hemorrhages. The cortices presented intense hyperemia in the lower layers and also small hemorrhages. There were no inflammatory lesions.

**CASE 4.**—The adrenal glands were completely destroyed by hemorrhages.

Microscopically, the lesions were almost the same in both glands. Each gland was narrow, with extremely intense hyperemia in the cortex, so that cortical tissue could not be seen, and it was impossible to distinguish whether red blood cells were in or outside the capillary walls. In the zona fasciculata there were several necrotic foci. The medulla had changed into a pool of blood. No inflammatory lesions were seen.

CASE 5.—Each adrenal gland showed hemorrhages, especially in the medulla. Microscopically, the glands were broad, with extraordinary hyperemia through the cortex and the medulla and multiple hemorrhages confluent in the medulla, more isolated in the cortical layers. Practically all tissue of both glands had been destroyed.

The examination of these adrenal glands revealed as a common characteristic of such cases hypoplasia of the adrenal glands (cases 1 to 4). In no case was thrombosis observed—neither in large veins nor in minor blood vessels—and the tables referring to this point published by Banks and McCartney<sup>7b</sup> are unconvincing. Neither have inflam-

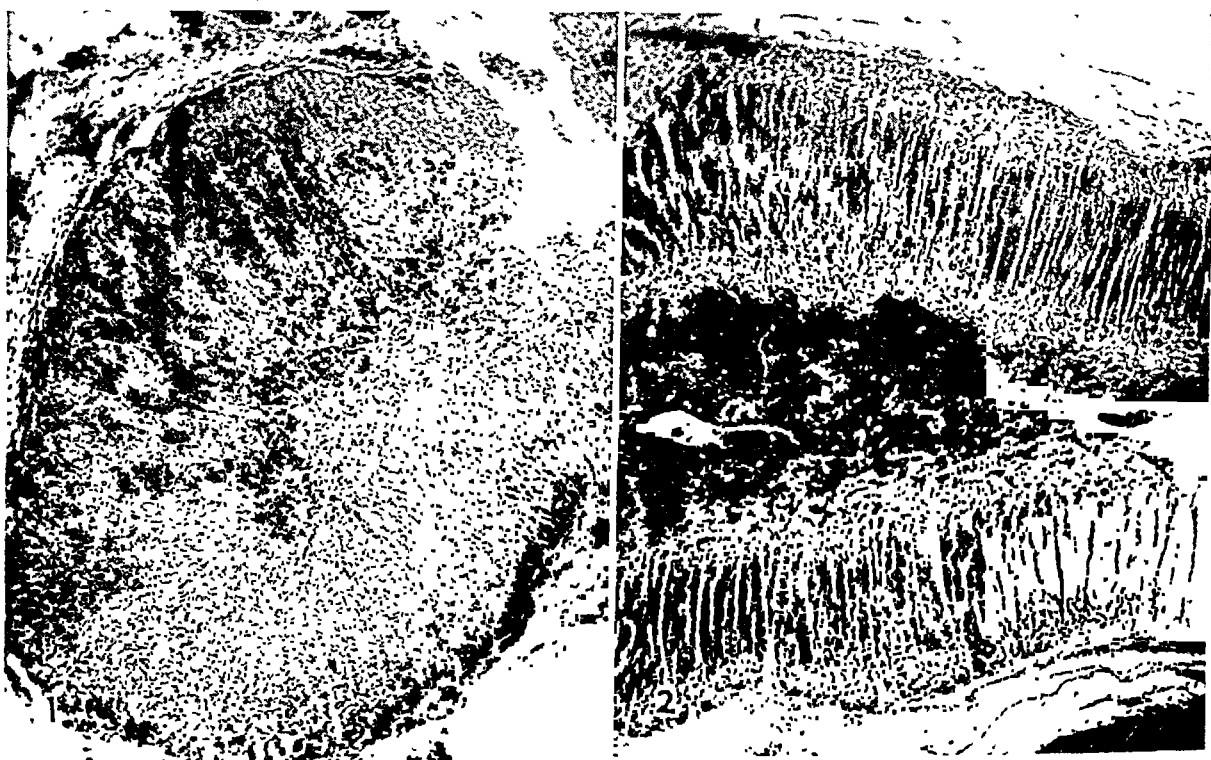


Fig. 1.—Intense dilatation of cortical capillaries with hemorrhage by diapedesis. No diffuse destruction is seen in this region, but illness was acute, lasting in all twenty hours. Hematoxylin-eosin;  $\times 23$ .

Fig. 2.—Right adrenal gland showing diffuse destruction of the medulla by hemorrhage. Note the normal structure of the cortical zone. The total duration of illness was twenty-nine hours. Hematoxylin-eosin;  $\times 23$ .

matory foci, described by the same authors, been observed in these 5 cases. Apparently there exist great differences in this matter. In my experience, lesions have been more serious in, and sometimes limited to, the medulla; this makes one suppose that lesions appear first in this part of the organ and later extend to the cortex. Usually cortical lesions are observed to be much less destructive than lesions of the medulla.

The macroscopic and microscopic appearances of the adrenal glands in these cases are impressive, but if one studies hormonal relations and realizes that the cortex, not the medulla, is indispensable for human life, one cannot feel satisfied with the simple explanation held forth in many papers on the subject, that in such cases sudden death is due to destruction of the adrenal glands (Ghon<sup>12b</sup>; Baumann<sup>12a</sup>; Levinson<sup>18</sup>). It is known that the cortex, not the medulla, is essential to human life, because substances similar to epinephrine can be produced in other regions. On the other hand, it is known that the medulla is a storehouse of the body's supply of epinephrine, and bleeding into the medulla might suddenly cause the entire stored amount of epinephrine to enter the circulation. In animals the injection of a large dose of epinephrine causes a short increase of pressure followed by severe and long hypotension (Grollmann<sup>19</sup>). Besides, there are relations between the thymus and the adrenal glands. Anatomically it may be stated that hyperplasia of the thymus is accompanied by hypoplasia of the adrenal glands. At all autopsies in the cases which I have observed there was marked hyperplasia of the thymus, and at the same time in the first 4 cases the adrenal glands were narrow. Maybe it is a coincidence but at any rate it is worthy of notice that in case 5, in which the weight of the thymus was lowest (15 Gm.), the thickness of the adrenal glands had not been reduced.

In none of these cases was it possible to weigh the gland as an index to its real volume, because the increase caused by hemorrhage would have completely falsified the result. Besides, in these cases the suprarenal glands were hard and dark red, and the surface of the incision was dry; it was impossible to squeeze out blood. This differs from the description of adrenal hemorrhage in the newborn, in which instead of the adrenal gland it is usual to find a "sac" of hemorrhagic contents.

#### COMMENT

Holmes and Cowan<sup>8</sup> observed in 3 cases edema of interstitial tissue, and they expressed the belief that death may be caused by circulatory collapse due to a serious decrease of the volume of blood. Martland<sup>14</sup> saw the principal cause of death in meningococcemia and not in destruction of the adrenal glands, but he did not make clear his opinion of the causes of the difference in clinical appearance and anatomic

18. Levinson, S. A.: J. Pediat. 14:506, 1939.

19. Grollmann, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936.

findings between cases of fulminant and cases of ordinary meningococcemia. The principal difficulty in the way of a logical explanation seems to be the question why just the adrenal vascular system should be so much affected in cases of the fulminant type; one might think of the predisposition of a pathologic gland in an organism of thymolymphatic constitution. Another factor could also influence adrenal localization: It is known that during the first months of life a physiologic transformation of the adrenal gland takes place (Dietrich and Siegmund <sup>20</sup>), and it is exactly in babies that the fulminant form of meningococcic sepsis is most frequently seen.

I believe that the fulminant septicemic syndrome with adrenal hemorrhages really forms a pathogenic entity. It is a serious infectious syndrome generally caused by *N. meningitidis* in a predisposed organism. There may be a predisposition in the thymolymphatic constitution combined with adrenal hypoplasia, which is also manifested in the pasty appearance of the children. (I can make statements about children only, as I have had no experience with adults.) Generally these children have little resistance against many and heterogenous agents (anesthetics, accidents, transfusions and punctures for example), and sudden death of these children is nothing new (Martland <sup>14</sup>).

If to this diminished resistance are added serious meningococcic infection and adrenal hemorrhages, a fatal ending cannot surprise one. Maybe the adrenal hyperemia—so frequently observed in autopsies during epidemic meningitis—can produce in a hypoplastic gland hemorrhages the intensity of which does not influence the clinical syndrome; in reality I can agree with Williams <sup>21</sup> and Thomas and Leiphart <sup>7b</sup> when they say that the clinical appearance is as serious in cases of unilateral or partial destruction of the adrenal glands as in cases of complete and bilateral destruction.

Naturally, therapy would be directed first against infection and secondary against possible circulatory failure due to adrenal destruction. This is based on the fact that in the experiment a complete lack of adrenal glands does not produce acute clinical symptoms during several days, so that lack of cortical hormone or of epinephrine is not the cause of this serious disease. Maybe it is a great quantity of epinephrine suddenly expelled into the blood stream: in that case, injections of epinephrine would surely not be indicated. The future will show whether this hypothesis is correct, whether in some cases hyperadrenalinemia could be established.

20. Dietrich, A., and Siegmund, H.: Die Nebenniere und das chromaffine System, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926.

21. Williams, H.: M. J. Australia 2:557, 1942.

SUMMARY

Adrenal hemorrhages were observed in 5 cases of fulminant meningococcic sepsis; the hemorrhages began in the medulla and occurred by diapedesis. Generally medullary lesions were much more extensive than cortical ones.

In the cases observed inflammatory lesions were not found in the adrenal glands, nor was thrombosis of the adrenal veins found. Definite hyperplasia of the thymus and hypoplasia of the adrenal glands occurred, which is interpreted as explained by the seriousness of the syndrome.

For historical reasons, the name "Waterhouse-Friderichsen syndrome" is not justified.

# RADIOIODINE AUTOGRAPHY IN STUDIES OF HUMAN GOITROUS THYROID GLANDS

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THE EFFECT of radioactive iodine on the photographic plate has made it possible to trace the deposition of the radioactive element in the thyroid gland. Moreover, this disposition may then be correlated with the histologic structure of the gland.<sup>1</sup> Employing the radioautographic technic, Leblond demonstrated that in rats most of the radioactive iodine, administered in varying doses,<sup>2</sup> was deposited in the colloid rather than in the cells<sup>3</sup> during periods of observation lasting thirty minutes to five days after intravenous injection of the iodine. A similar preferential distribution was shown in pituitary-treated and in hypophysectomized animals.<sup>4</sup> Whatever the condition of the thyroid gland, the radioiodine was more abundant in the acidophilic colloid than in the basophilic colloid,<sup>5</sup> perhaps owing to a more rapid turnover of iodine in the latter.<sup>4</sup> Following the injection of a single large dose of iodine (1 mg.), when the iodide ion was present as such in the rat's gland,<sup>6</sup> the autographs showed diffuse pictures even after freezing-drying fixation. This suggested that either the iodide ion was displaced during the paraffin embedding or that the picture obtained was truly representative of the diffuse distribution of the iodide throughout the epithelium and the colloid.<sup>3a</sup>

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1. Hamilton, J. G.; Soley, M. H., and Eichorn, K. B.: Univ. California Publ., Pharmacol. (no. 28) **1**:339, 1940. Gorbman, A., and Evans, H. M.: Proc. Soc. Exper. Biol. & Med. **47**:103, 1941.

2. Leblond, C. P.: J. Anat. **77**:149, 1943.

3. Leblond, C. P.: (a) Stain Technol. **18**:159, 1943; (b) footnote 2.

4. Leblond, C. P.: Anat. Rec. **88**:285, 1944.

5. Footnotes 2 and 3.

6. Leblond, C. P.: Am. J. Physiol. **134**:549, 1941.

By the autographic method, Hamilton, Soley, Reilly and Eichorn<sup>7</sup> were able to study the deposition of radioactive iodine in human thyroid glands. They found that in goitrous glands the element was in general concentrated in the most actively functioning parts of the glands.<sup>7a, c</sup> In normal thyroid tissue the radioactive iodine was evenly distributed throughout the section. In nontoxic goiter<sup>7a, c</sup> and in goiter accompanied by severe hypothyroidism<sup>7b</sup> the new iodine was accumulated in the cellular portions of the gland but not in the colloid. On the other hand, in hyperplastic thyroid tissue the colloid was found to have a higher proportion of accumulated radioactive iodine than the cells of the adjacent acini, and this was explained as due apparently to the rapid outflow of this material from the hyperplastic cells.<sup>7a, c</sup> Cancer tissue was found to have no apparent ability to store administered radioactive iodine.<sup>7a, c</sup>

While the metabolism of radioiodine was being investigated in human thyroid glands,<sup>8</sup> removed at operation, autographic studies were undertaken to correlate, if possible, the thyroid responses revealed by chemical analyses to the distribution of iodine in the thyroid glands. Likewise, the making of radioautographs of human thyroid glands, with Leblond's technic,<sup>9</sup> was instituted to determine whether the administered radioiodine was to be found essentially in the colloid, as noted by Leblond in animal glands at varying intervals of time<sup>9</sup> and by Hamilton and his group in human hyperplastic thyroid tissue,<sup>7a, c</sup> or essentially in the cells, as observed by Hamilton in nontoxic goiters with<sup>7a, c</sup> or without<sup>7b</sup> accompanying hypothyroidism.

#### METHODS

Radioiodine containing 25 to 1,000 microcuries of the radioelement I<sup>131</sup> and about 2 micrograms of iodine, I<sup>127</sup>, was administered orally to R. D. and E. S. fifteen and twenty hours, respectively, before thyroidectomy. Since the radioactive iodine was administered by mouth, this dose might be considered as a physiologic amount of iodine. Autographic studies were carried out with blocks of the extirpated goiters. Bouin and Susa fluids were used for fixation. The embedding was usually done with paraffin, although in 1 case satisfactory results were obtained by mounting a whole lobe of the gland in celloidin (a concentrated preparation of pyroxylin). The various technics were speeded up by using small amounts of tissue whenever possible. When a whole lobe was included in celloidin, sections could be obtained in six days by combined use of a vacuum during clearing and the heat of a 37 degree oven during the infiltration with celloidin.

7. (a) Hamilton, J. G.: Radiology 39:541, 1942. (b) Hamilton, J. G.; Soley, M. H.; Reilly, W. A., and Eichorn, K. B.: Am. J. Dis. Child. 66:495, 1943. (c) Hamilton, Soley and Eichorn.<sup>1</sup>

8. Leblond, C. P.; Poppel, I. D.; Riley, E.; Radike, M., and Curtis, G. M.: Radio-Iodine and Iodine Fractionation Studies of Human Goitrous Thyroids, J. Biol. Chem., to be published.

The cut sections were affixed to the slide with the help of a 0.5 per cent solution of celloidin, and the slides were then placed in 70 per cent alcohol for a few minutes and dried. The dry slides bearing the sections were placed on the sensitive face of photographic plates (Eastman Kodak contrast lantern slide plate) in the dark room and left there for about two to three weeks. At the end of this period the plates were developed in fine grain developer and fixed as usual. The histologic slides were stained either by Masson's trichrome technic or by the hematoxylin-eosin method. The reactions on the plates were compared with

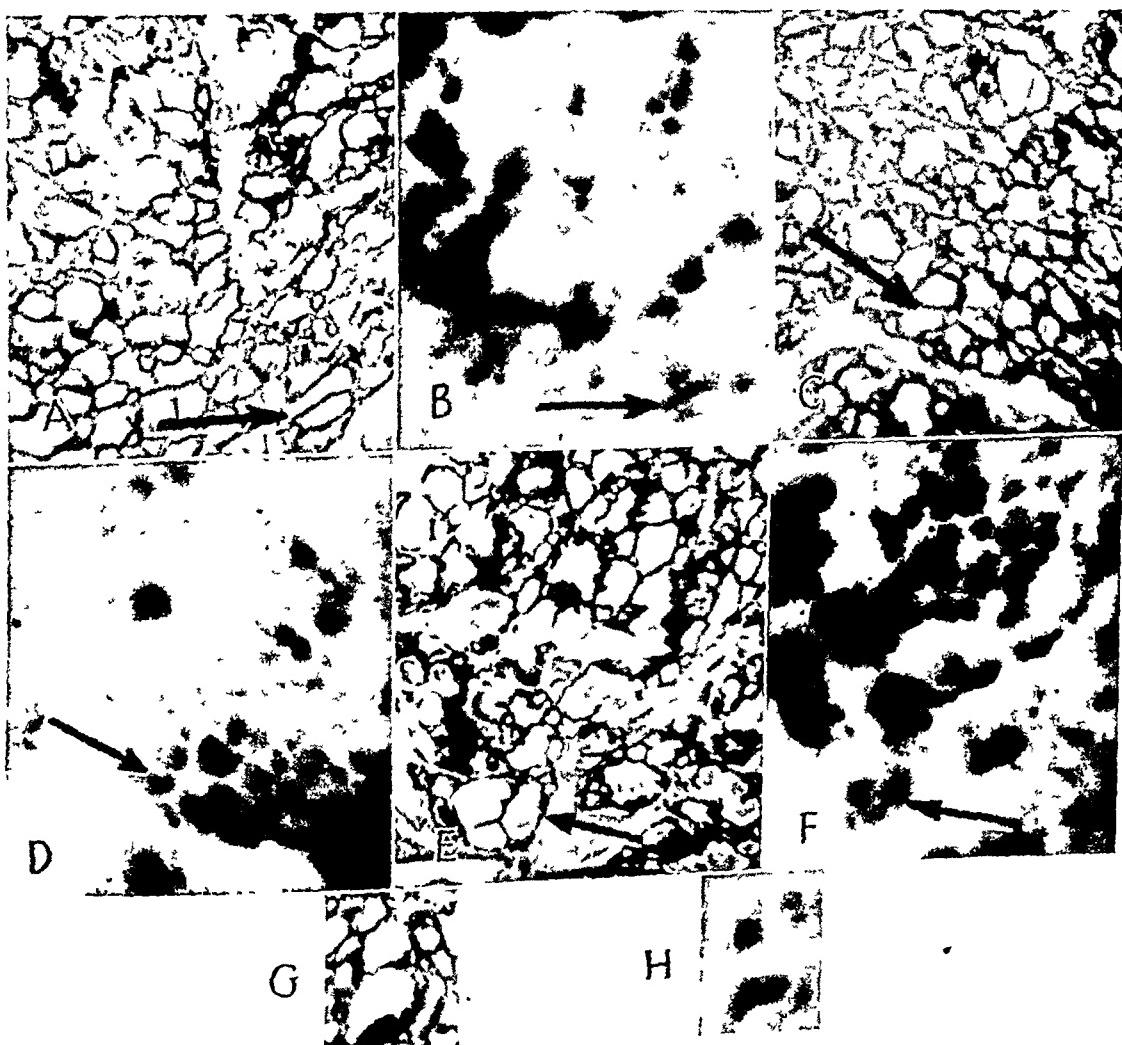


Fig. 1.—Sections of thyroid gland of a patient (E. S.) with nontoxic diffuse colloid goiter and a basal metabolic rate of minus 7 per cent, and the corresponding radioiodine autographs. A comparison of the photomicrographs (A, C, E and G) and the radioautographs of these sections (B, D, F and H) demonstrates that the radioiodine is deposited in the colloid. This remarkable feature is reemphasized when the arrow-indicated follicles of A, C and E are compared with the arrow-indicated radioautographic areas of B, D and F. Note that the radioautographic areas are almost similar in configuration to the colloid areas present. Note also that the vacuole in section G shows practically no radioactivity in H.

the histologic slides with the help of a dissecting microscope. Photographs were made of the plates (autographs) and slides at the same magnification.

It was soon found that very large tracer doses of radioactive iodine must be used to obtain satisfactory autographs from human thyroid glands. Only after administration of about 500 microcuries or more was it possible to obtain autographs sufficiently intense to lend themselves to profitable study. The autographs shown

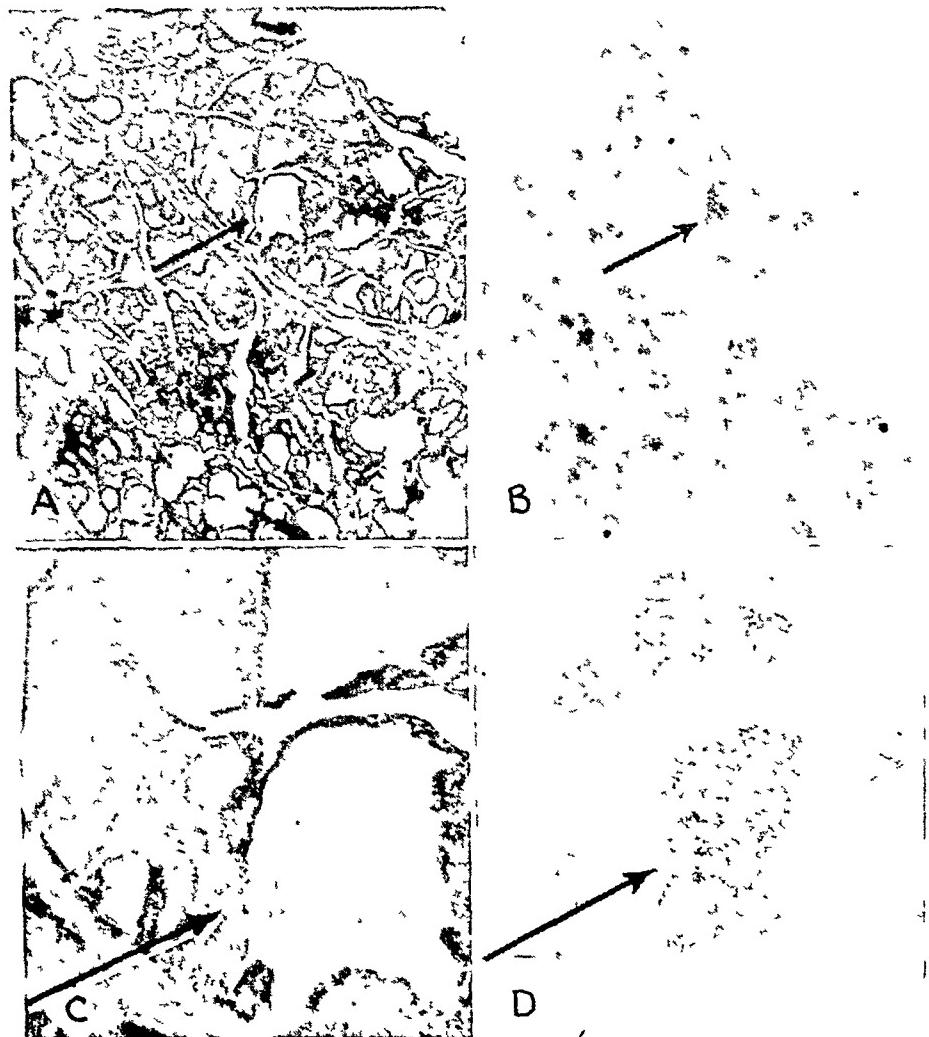


Fig. 2.—Sections of the thyroid gland of a patient (R. D.) with exophthalmic goiter and a basal metabolic rate of plus 40 per cent, and the corresponding radio-iodine autographs. A comparison of the photomicrographs (*A* and *C*), which are typical of toxic diffuse hyperplastic goiter, and the radioautographs of these sections (*B* and *D*) again emphasizes that the radioiodine is deposited in the colloid. This is especially clear when comparing the arrow-indicated areas.

here represent studies of the thyroid gland of a patient (E. S.) with nontoxic diffuse colloid goiter and a basal metabolic rate of minus 7 per cent (fig. 1) and of a patient (R. D.) with moderately active exophthalmic goiter and a basal meta-

bolic rate of plus 40 per cent (fig. 2). The protocols of these patients have been presented in another communication.<sup>9</sup>

#### HISTOLOGIC RESULTS

Analysis of the autographs revealed that only true thyroid tissue affected the photographic plate. As in the case of the thyroid glands of rats,<sup>3</sup> the blood vessels, the nerves and the connective tissue stroma of the human thyroid glands contained no detectable radioiodine. Whether in diffuse colloid or in hyperplastic goitrous tissue, the autographic reactions were revealed as spots corresponding in size and shape to the colloid accumulations. In no instance did the groups of cells contain any detectable iodine by this method (figs. 1 and 2).

Examination of the reactions produced by the colloid showed that these varied in intensity in neighboring follicles (figs. 1 and 2). On the other hand, in any given follicle the reaction was fairly homogeneous, suggesting that each part of the colloid of one follicle contained the same amount of radioactivity (figs. 1 and 2). There was, however, an exception in the case of the single large vacuoles which were sometimes found in the large follicles. Such vacuoles did not affect the photographic plate and appeared as white spots in the dark area produced by the colloid present in a follicle (fig. 1, G and H).

#### COMMENT

These autographs demonstrate that most of the radioactive iodine present in diffuse colloid goiter as well as in exophthalmic goiter about twenty hours after its administration was deposited in the colloid of the thyroid follicles. If the cells of the follicles contained some radioiodine, also insoluble to the histologic reagents, it was apparently only a small proportion of that within the gland, since there was not enough to affect the zone of the photographic plate facing the cell accumulations.

Whatever iodine was in soluble form in the thyroid gland should have been extracted during the preparation of histologic sections since the fixatives used were alcohol, xylene and paraffin. The inorganic iodine should have been removed, therefore, while the iodine of thyroglobulin should not have been affected. In both the diffuse colloid goiter and the exophthalmic goiter less than 10 per cent of the radioactive iodine was in the inorganic form.<sup>8</sup> The rest was incorporated in diiodotyrosine or thyroxin.<sup>8</sup> It may be estimated that about 90 per cent of the radioiodine revealed by the autographic method was present as diiodotyrosine. Thus, a comparison of the chemical<sup>8</sup> and histologic findings indicates that most of the radioiodine passed through the epi-

9. Puppel, I. D.; Leblond, C. P.; Riley, E., and Curtis, G. M.: The Clinical Significance of the Functional Behavior of Adenomas of the Thyroid Gland, *J. Lab. & Clin. Med.*, to be published.

thelium of the thyroid follicle to be deposited as diiodotyrosine in the colloid.

The question arises why somewhat different results have been obtained in these autographs of human thyroid glands and those of Hamilton and his group.<sup>7</sup> In our investigation the radioiodine, regardless of the type of goitrous tissue, was always well defined in areas of colloid. In Hamilton's material,<sup>7a,c</sup> on the other hand, where localization of radioiodine often appeared less sharply circumscribed in the follicles it nevertheless showed a preferential distribution for certain tissues. Thus the amount of radioiodine was greater in tissue areas of greatest activity, a fact corroborated by our chemical findings,<sup>8</sup> which show a lesser fixation of radioiodine in thyroid adenomas than in the surrounding thyroid tissue, which is presumably more active.

These differences in autographic results cannot be explained at this time. Can they be due to differences in method, dosage, reagents, time factors or strength of radioactivity used? Further investigation is necessary.

Finally, it should be noted that the radioiodine is homogeneously distributed throughout the colloid in any given follicle, since many histologists believe that there may be several kinds of colloid or colloid-like material in a given follicle. Our result suggests, on the contrary, that there is probably no difference between the colloid in the center of a follicle and that at the periphery. Material entering the colloid is apparently homogeneously distributed.

#### SUMMARY

Radioiodine was administered orally to patients with diffuse colloid and exophthalmic goiter before subtotal thyroidectomy. Subsequent autographic study of the removed glands showed that the iodine was rapidly deposited in a stable form in the colloid part of the thyroid follicle.

## HEPATIC AND RENAL NECROSIS IN ALLOXAN DIABETES OF RABBITS

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WITHIN the three years that have elapsed since Dunn, Sheehan and McLetchie<sup>1</sup> first reported selective necrosis of the islets of Langerhans in rabbits given injections of alloxan, the literature has been flooded with pertinent articles. As the published material has already been reviewed several times,<sup>2</sup> it would serve no useful purpose to summarize it again now. Suffice it to say that the administration of alloxan is an effective and convenient means of producing permanent diabetes in many animals, with the result that the problem of diabetes itself can be studied experimentally with greater facility.

The necessary amount of alloxan has been carefully investigated by several experimenters, and at the present time it appears to be the consensus that in the rabbit an effective diabetogenic dose is 200 mg. of a 4 or 5 per cent solution administered intravenously.<sup>3</sup> Smaller doses, even if injected repeatedly, may not be followed by permanent diabetes,<sup>4</sup> and larger doses may result in death of the animal in a few hours or in severe necrosis of the epithelium of the renal tubules.<sup>5</sup> In an attempt to produce intercapillary glomerulosclerosis in rabbits we used the accepted method as outlined only to find that although most of the animals survived the hypoglycemic stage, many of them died in the next few days in uremia. Necropsy disclosed extensive necrosis and sometimes calcification of the epithelium of the renal tubules, partial or complete necrosis of the islets of Langerhans including not only the beta

1. Dunn, J. S.; Sheehan, H. L., and McLetchie, N. G. B.: Lancet **1**:484, 1943.

2. (a) Duff, G. L., and Starr, H.: Proc. Soc. Exper. Biol. & Med. **57**:280, 1944. (b) Gomori, G., and Goldner, M. G.: ibid. **54**:287, 1943. (c) Bailey, O. T.; Bailey, C. C., and Hagan, W. L.: Am. J. M. Sc. **208**:450, 1944. (d) Joslin, E. P.: New England J. Med. **230**:425, 1944.

3. Gomori and Goldner.<sup>2b</sup> Bailey and co-workers.<sup>2c</sup>

4. Bailey, C. C.; Bailey, O. T., and Leech, R. S.: New England J. Med. **230**:533, 1944.

5. (a) Goldner, M. G., and Gomori, G.: Endocrinology **33**:297, 1943. (b) Brunschwig, A., and Allen, J. G.: Cancer Research **4**:45, 1944.

cells but the alpha cells, and massive necrosis of the peripheral portions of the hepatic lobules. We record these results because the extensive renal changes occurred in many of our animals with a dose of alloxan that should be relatively harmless to the kidneys and because a thorough search of the literature discloses only one report of focal hepatic necrosis observed in rats treated with alloxan<sup>2b</sup> (in 2 of 49 given injections) and no reports of massive necrosis observed in the many animals used in such experiments.

#### MATERIAL AND METHOD

Each of 30 rabbits weighing between 2,150 and 3,560 Gm. received a single intravenous injection of a 4 per cent solution of alloxan in distilled water in an amount equivalent to 200 mg. per kilogram of body weight. The animal was allowed plenty of water and food in the form of pellets and greens for twenty-four hours before and after the injection. The blood levels of nonprotein nitrogen and glucose were determined before the experiment was started, and the latter was determined at frequent intervals during the first twelve hours after the injection. Subsequently it was determined daily during the first few days and then at weekly intervals. The blood nonprotein nitrogen level was first redetermined on the fourth day after several rabbits died in spite of relatively normal blood sugar levels. Thereafter the nonprotein nitrogen level was determined at sporadic intervals and at the time of death in all instances. An analysis of urine collected from the bladder was made to the time of necropsy. Hypoglycemic convulsions were treated with 3 to 5 cc. of 50 per cent dextrose, administered intravenously, and subsequent hyperglycemia was controlled first with insulin injection U. S. P. and after the first week with protamine zinc insulin. Two rabbits not treated with alloxan each received 10 cc. of a 50 per cent solution of dextrose intravenously on three successive days. Tissues were fixed in Helleys fluid and solution of formaldehyde U.S.P. diluted 1:10, and were stained with hematoxylin and eosin, Bensley's stain for alpha and beta granules in the islets of Langerhans, nile blue sulfate stain for fat in the liver and the kidneys and Best's carmine stain for glycogen in the liver and the kidneys.

#### CLINICAL OBSERVATIONS

The normal nonfasting blood sugar levels ranged from 105 to 151 mg. per hundred cubic centimeters and the nonprotein nitrogen levels from 41 to 58 mg. per hundred cubic centimeters. After the administration of alloxan there was initial hyperglycemia, the blood sugar rising as high as 500 mg. per hundred cubic centimeters which was followed by hypoglycemia with a blood sugar level of 30 mg. per hundred cubic centimeters or less, and subsequently by permanent hyperglycemia, with the level of blood sugar as high as 800 mg. per hundred cubic centimeters. In 10 rabbits hypoglycemic convulsions did not develop, whereas 20 rabbits were convulsed from one to five times. The convulsions first occurred as early as three hours and as late as sixteen hours after the injection of alloxan. Nine rabbits died of the initial hypoglycemia, while 21 survived, and in each of these diabetes developed. Sixteen of the 21 survivors died at sporadic intervals of from three days to thirty days after the injection, and 5 are still living.

Most of the animals that died within the first week refused to eat or drink and became drowsy and comatose before death. Although their blood sugar levels ranged from 230 to 500 mg. per hundred cubic centimeters, the urine of none of

the rabbits showed acetone or diacetic acid. The blood nonprotein nitrogen levels of the rabbits that died within the first two weeks of the experiment ranged from 130 to 342 mg. per hundred cubic centimeters, and those of the rabbits that succumbed after that time ranged from 100 to 160 mg. per hundred cubic centimeters. Most of the latter died in hypoglycemia from overdoses of insulin. Three of the rabbits with a greatly elevated blood level of nonprotein nitrogen had no urine in their bladders at the time of necropsy, and another had only about 4 drops present. In each of these instances the cages were dry. Analysis of the few drops of urine from the rabbit just referred to, and of urine from other rabbits, disclosed a specific gravity as high as 1.036, an acid or a neutral reaction, a cloud to a heavy precipitate of albumin, sugar as much as 1 per cent in those treated for hypoglycemia and microscopically leukocytes, erythrocytes, cylindriform and hyaline, granular or cellular casts. None of the urines contained acetone or diacetic acid.

#### PATHOLOGIC OBSERVATIONS

*Macroscopic Changes.*—There were no noteworthy gross changes in any of the organs. In some instances the kidneys were entirely normal, in others they were congested, and in others still they were considerably paler than normal. The capsules were not adherent, and in some cases the cut surfaces of the kidneys showed only slight radial streaking. The liver revealed no appreciable change in color, although in some cases it was distinctly softer than normal. None of the animals was jaundiced. The pancreas was sometimes not as pink as it is normally, often considerably softer and sometimes less bulky, so that occasionally one could not be certain that one was not handling a piece of fat instead of the pancreas. Cataracts were not seen in any of the animals, and the remaining organs showed no contributory changes.

*Microscopic Changes.*—(a) Pancreas: The islets of Langerhans showed changes progressing from degeneration to complete necrosis and disappearance. At five hours they were swollen and appeared more conspicuous than those in a normal organ. The cytoplasm was homogeneous, stained deep pink and tended to coalesce, thus obliterating the cell boundaries. Most of the nuclei were pyknotic, although a few had undergone rhesis. Generally the process was diffuse and involved both the alpha and the beta cells. Unscathed cells when present, however, were found at the periphery. The changes increased in severity so that within twelve hours there were notable shrinkage in size, fading or complete disappearance of the nuclei, and granularity of the cytoplasm. Within twenty-four hours most of the islets were completely necrotic (fig. 1 A) and further reduced in size, although there were generally some in which a few peripheral cells remained intact. From the third to the ninth day there were various mixtures of degeneration, necrosis and disappearance of the islets with, however, always a few present that were less damaged. Numerous sections disclosed an unequivocal paucity of islets and what appeared to be gradual replacement of the disappearing elements with pancreatic cells of an exocrine variety (fig. 1 B). These cells were seen to be intimately connected with the surrounding acinous cells. The younger ones were somewhat smaller, and their cytoplasm was more homogeneous, but as they aged, they became fewer and fewer and more and more of them disclosed granularity of cytoplasm until ultimately they became indistinguishable from the surrounding acinous cells. By the fourteenth day there were many instances in which no vestige of any islet could be found in the pancreas. By the twenty-first day, however, sections of two organs did show a fair number of small but relatively normal islets. It could not be ascertained whether these were regenerated or originally

undamaged structures. In no instance did the pancreas show hyalinization, leukocytic infiltration, mitosis or damage of the exocrine portion of the gland.

(b) Kidneys: Within five hours after the injection of alloxan the epithelium of all the proximal convoluted tubules of the affected kidneys was swollen to such a degree that the lumens were completely obliterated. The cytoplasm was granular, and the nuclei were already poorly stained, rhectic or entirely absent. There was granular material within the lumens of the more distal portions of the nephrons,

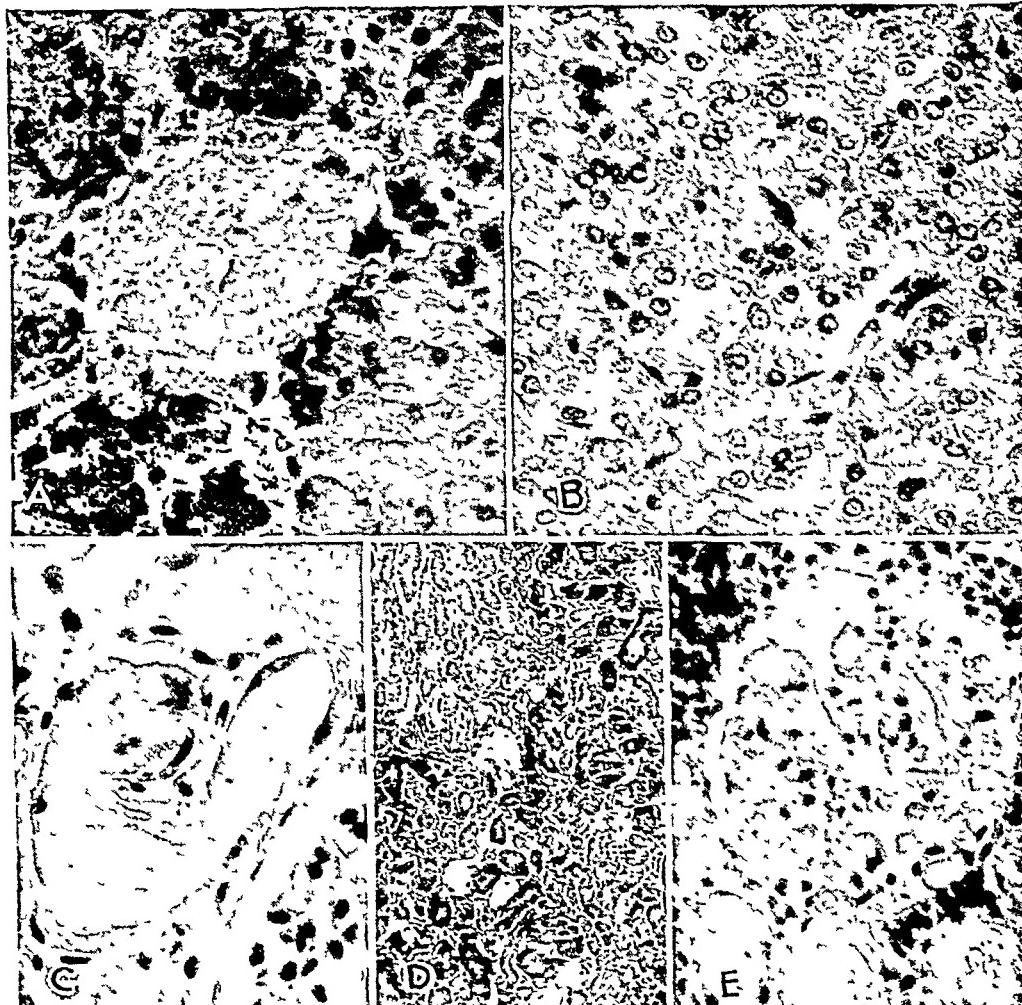


Fig. 1.—*A*, section of pancreas from a rabbit that died fourteen hours after an injection of alloxan, showing complete necrosis of an islet. Hematoxylin and eosin;  $\times 400$ .

*B*, section of pancreas showing complete replacement of a necrotic islet with newly proliferated cells of an exocrine variety. Their nuclei are similar to those of the surrounding cells, but their cytoplasm is more homogeneous and less granular. Hematoxylin and eosin;  $\times 400$ .

*C*, section of kidney showing necrosis of a proximal convoluted tubule and regeneration of the epithelium at the periphery. Hematoxylin and eosin;  $\times 400$ .

*D*, section of kidney showing calcification of the necrotic epithelium of the proximal convoluted tubules. Hematoxylin and eosin;  $\times 75$ .

*E*, section of kidney showing necrosis of a glomerulus and of the tubules and densely infiltrating polymorphonuclear leukocytes. Hematoxylin and eosin;  $\times 400$ .

but the remaining structures were normal. Within fourteen hours the process was more advanced, the tubular epithelium in focal areas being completely necrotic and lying free in the lumen. At approximately the same time some of the epithelial cells became irregularly vacuolated, and the tubular lumens contained granular, hyaline and erythrocytic casts. At nineteen hours there was severe diffuse necrosis of practically all of the proximal convoluted tubules, and at thirty hours calcification of some of the necrotic tubules was already present. At three days in some cases the kidneys disclosed edema of the interstitial connective tissue, scattered collections of leukocytes and elongated, flattened cells at the periphery of the necrotic material within the tubules. These regenerating cells were more conspicuous by the fourth day (fig. 1 C). At this time vacuoles were also present in the less disturbed cells, and sections stained with nile blue sulfate revealed numerous blue to purple granules within both the tubular epithelium and the glomeruli. By the seventh day many of the tubules showed degeneration and necrosis, but the most striking change was the extensive calcification of the proximal convoluted tubules (fig. 1 D). Foci of leukocytes were observed throughout the cortex. Beyond the seventh day the changes were less spectacular, the kidneys being relatively normal in most cases. Hyaline casts were still present in the distal convoluted and collecting tubules. Here and there were a few foci of necrotic condensed tubules, surrounded by a few lymphocytes and plasma cells. In some sections there were only a few calcified tubules, with remaining elements relatively normal. The nile blue sulfate stain revealed no fat, and Best's carmine stain showed a few droplets of glycogen in some of the arched tubules of the rabbits whose diabetes was not completely controlled.

One rabbit that died at eleven hours deserves special mention, for sections of the kidneys showed almost complete necrosis of not only the tubules but the glomeruli as well. The tubular epithelium was granular and its inner border fuzzy. The nuclei were poorly stained or entirely absent, and the tubular lumens contained debris and erythrocytes. Similar cytoplasmic and nuclear changes were present in the glomeruli. There was extensive interstitial edema, and there were focal collections of neutrophils around many glomeruli and tubules (fig. 1 E).

Not all rabbits showed the changes described in the foregoing paragraphs. Among the 25 animals that died there were 14, or 56 per cent, that disclosed tubular necrosis, and in 8 of these 14, or 32 per cent of the total number of 25, the necrosis was diffuse and in 6, or 24 per cent of the total, it was focal.

(c) Liver: Within five hours after the injection of alloxan sections of the liver disclosed diffuse coagulative degeneration to necrosis of the peripheral two thirds of the hepatic lobules (fig. 2 A). The demarcations between the healthy and the affected zones were not sharp. In the latter the cells were greatly swollen; their boundaries were indistinct; their cytoplasm was finely granular and pink; their nuclei were shrunken and pyknotic, with lighter staining or with staining entirely absent, and prominent capillaries contained an excess of neutrophils. At ten hours the changes were essentially the same except that the cytoplasm of the cells nearer the portal radicles first became vacuolated and then completely disintegrated, so that there were definite areas of complete necrosis. At the junction of the normal and the necrotic tissue there was a conspicuous zone of polymorphonuclear leukocytes (fig. 2 B). Similar but somewhat more extensive changes were encountered in the livers of the animals dying within the first four days. In 2 rabbits that died on the fourth day there was not only necrosis of the peripheral portions of the lobules but marked deposition of fat within the centralmost portions (fig. 2 C). In a few of the animals that died after the seventh day there was

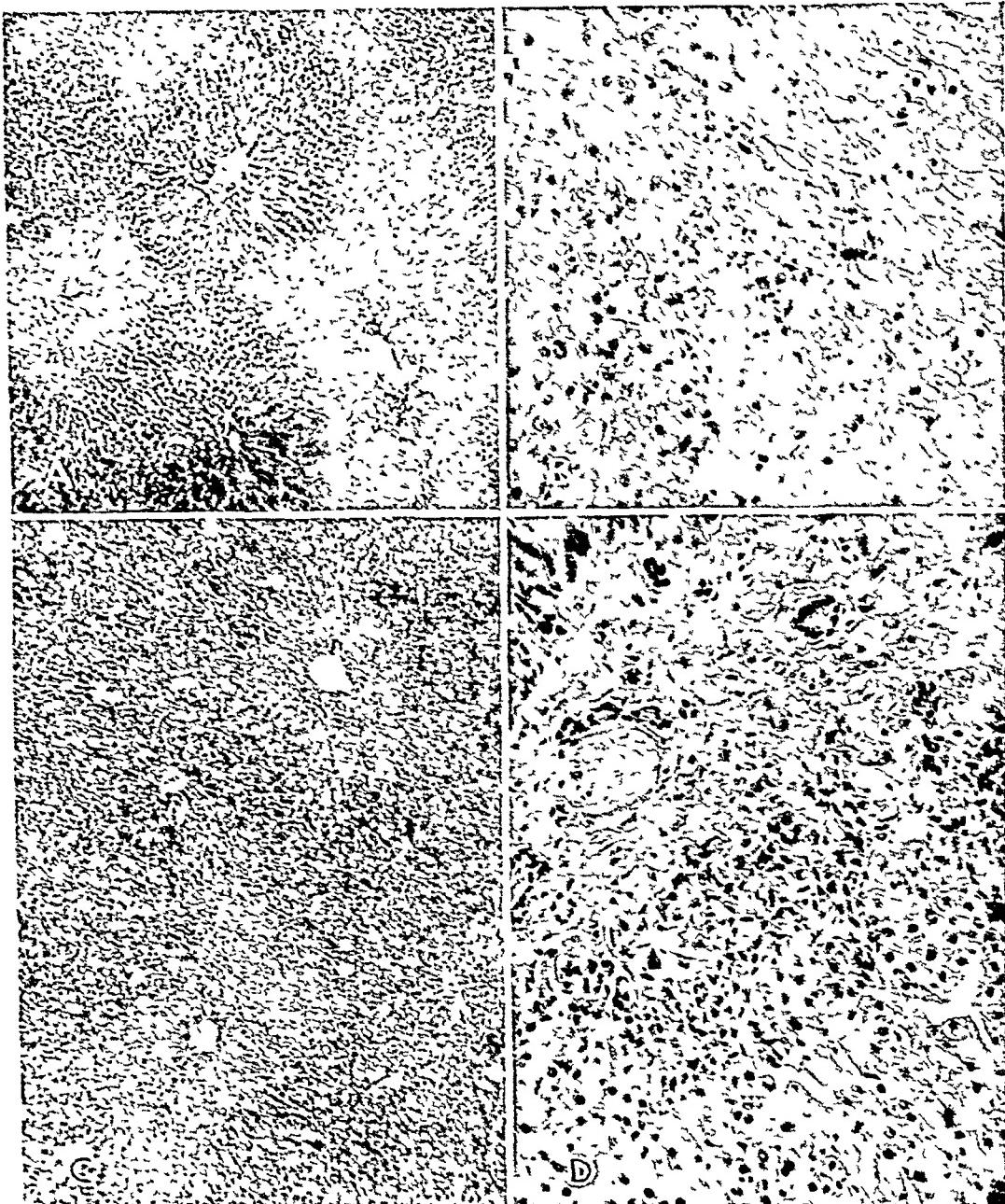


Fig. 2.—*A*, section of liver showing coagulative necrosis of the peripheral portions of the hepatic lobules. Hematoxylin and eosin;  $\times 50$

*B*, section of liver showing liquefactive necrosis, leukocytic infiltration and early fatty metamorphosis of otherwise relatively normal central liver cells. Hematoxylin and eosin.  $\times 200$ .

*C*, section of liver showing periportal necrosis and marked fatty metamorphosis of the central portions of the lobules. Hematoxylin and eosin;  $\times 50$

*D*, section of liver showing proliferating hepatic cells invading recent, loose, connective tissue in a portal radicle. Hematoxylin and eosin;  $\times 200$ .

similar central deposition of fat without, however, peripheral necrosis of the lobules. With nile blue sulfate, these deposits stained blue to purple. The livers of most of the rabbits that died after the seventh day had an essentially normal histologic appearance, with a return of the normal glycogen content, which during the stage of necrosis was completely or almost completely depleted.

Sections of the liver of 1 animal that died sixteen days after the administration of alloxan showed rather prominent portal radicles (fig. 2 D). These contained the normal portal triads, which were surrounded, however, with an abundant amount of recent, loose connective tissue. The liver cells adjacent to these areas were smaller than normal; their cytoplasm was deep pink, and their nuclei were relatively large. They were irregularly arranged, and although directly connected with the adjacent liver cell cords, they did not form a continuation of the regular lobular pattern. They appeared to be in the process of proliferation and to be invading the portal radicles, where they often produced new bile ducts.

As in the kidneys, the changes mentioned were not present in the livers of all the animals that died. When encountered, however, the process was diffuse; that is, it involved all portions of the liver with equal severity. Of the 25 animals that died, hepatic necrosis was found in 10, or 40 per cent, and was present in all but 1 of the animals that died within the first four days. Fatty metamorphosis was encountered in 6 rabbits, or 24 per cent, and was found as late as the thirtieth day after injection.

Sections of other organs, including the adrenal glands, showed no pertinent changes.

#### COMMENT

The response of the rabbits to alloxan—temporary hyperglycemia and hypoglycemia followed by permanent hyperglycemia—was no different from that in other experiments already published. The explanations for this typical curve have also been adequately covered<sup>6</sup> and will not be repeated here. The initial hyperglycemic stage is of little consequence, for it does no harm, but the hypoglycemic stage is serious; although it is only temporary, it may last from a few to twenty-four hours and in spite of the administration of dextrose may prove fatal to the animals. Some authors have intimated that a single intravenous injection of dextrose solution will tide the animals over this period.<sup>2c</sup> We used as many as a dozen injections, and still the animals became convulsed, indicating that constant supervision is necessary for at least thirty hours after an injection of alloxan. Signs of impending hypoglycemic convulsions were noted in not a few of the rabbits. The animals appeared rather apprehensive, nervous and overly alert. Their posture was usually that of sitting with the front legs stretched to a maximum and the neck arched backward. Their heads moved backward and forward in short, jerky, purposeless movements, and they ground their teeth vigorously. When dextrose was not administered, generalized convulsions usually ensued in from fifteen to sixty minutes.

6. Hughes, H.; Ware, L. L., and Young, F. G.: Lancet 1:148, 1944. Goldner, M. G., and Gomori, G.: Endocrinology 35:241, 1944. Hard, W. L., and Carr, C. J.: Proc. Soc. Exper. Biol. & Med. 55:214, 1944.

Diabetes developed in all of our rabbits that survived the initial hypoglycemia. For its control we used protamine zinc insulin, administered once daily. Unlike man, rabbits are most difficult to stabilize, probably because of the irregular intake of food. We found it not too infrequent that animals receiving 1 unit of insulin regularly would have high blood sugar levels, 400 to 500 mg. per hundred cubic centimeters, for several days or even weeks and then with continuation of the same regimen hypoglycemic convulsions would suddenly develop and many of the rabbits would die. It would be ideal to determine blood sugar daily and administer the insulin accordingly. This, however, we found impracticable, for it was not long before the veins of the ears were so destroyed that to get enough blood for even a weekly microdetermination of sugar was often difficult.

The changes in the islets of Langerhans have been described minutely several times.<sup>7</sup> Most authors have noted that the central portions of the islets are attacked first, the peripheral cells being left intact, and that only the beta cells are disturbed, while the alpha cells remain unscathed. Although this was true also in those of our animals that died within a few hours after the injection of alloxan, in those whose death was delayed there was complete necrosis of all the islet cells. Mitosis or hyalinization of the islets was not observed in any case. There was, however, complete disappearance of the necrotic material, so that in a few cases not a single islet of Langerhans could be found. This process was gradual, and it appeared to consist not merely of absorption of the debris and collapse of the "unsupported" sides but of actual proliferation of the adjacent acinous cells. All stages could be traced from the appearance of small dense cells in direct continuation with the pancreatic acini to their gradual enlargement and maturation until they became indistinguishable from the regular exocrine cells. Simultaneously they gradually encroached on the remains of the islets until the detritus was completely replaced with the newly formed cells. Although the islets of Langerhans were more numerous in the animals that died after the eighteenth day than they were in those that died sooner, it could not be ascertained whether there was proliferation of the islet cells or whether in these animals all the islets were not initially destroyed.

The changes in the kidneys bore a striking resemblance to those observed in mercury bichloride poisoning. There is no question that uremia induced by the extensive tubular necrosis was the cause of death in at least 8 animals. This side effect, therefore, cannot be overemphasized. That in many of the surviving animals probably similar but perhaps not quite as extensive renal changes developed with recovery, is evidenced

7. Dunn, J. S.; Kirkpatrick, J.; McLetchie, N. G. B., and Telfer, S. V.: *J. Path. & Bact.* **55**:245, 1943. Gomori and Goldner.<sup>2b</sup> Bailey and co-workers.<sup>2c</sup>

by the obvious regeneration of the tubular epithelium occurring at a time when the necrosis was still apparent and by the calcification still present in the proximal convoluted tubules of the kidneys of 1 rabbit that died thirty days after the injection of alloxan. Except for these calcific deposits the rest of the renal structure was entirely normal, indicating that regeneration was complete.

The alterations in the liver were diffuse and appeared to consist of four distinct processes. First, there was general depletion of the glycogen content of the liver. Second, there was initially coagulative and then liquefactive necrosis of the peripheral portions of the hepatic lobules with often a zone of inflammation along the margin of the dead tissue. This perilobular involvement was similar to that seen in women in eclampsia and in animals following intravenous injection of chloroform.<sup>8</sup> Third, there was severe fatty metamorphosis of the central portions of the lobules, which first became manifest while the necrosis was still apparent. Whereas fatty changes in the liver have been noted by several observers,<sup>9</sup> massive hepatic necrosis has not been previously reported. Fourth, there was some evidence of healing in 1 rabbit that died in hypoglycemia sixteen days after the injection of alloxan. The portal radicles were prominent and filled with loose, recent fibrillary connective tissue. Encroaching on and gradually infiltrating this were evident young, regenerating liver cells and newly formed bile ducts, resulting in the formation of early portal cirrhosis.

Finally, while extensive necrosis was observed in the pancreas, the kidneys and the liver, there was no regularity of involvement of these organs. None was necessarily affected to the exclusion of the others, nor was each equally involved in all animals. In all three organs the necrosis was probably caused by alloxan or a derivative of alloxan acting directly on tissue cells. The specific manner in which the agent affects the islets of Langerhans is most obscure. It might act on the liver cells during the process of detoxification, if the liver acts in this capacity, and it might act on the tubular epithelium of the kidneys while being excreted. As far as we know, however, there is no adequate proof for either hypothesis.

#### SUMMARY

Thirty rabbits received a single intravenous injection of 200 mg. of alloxan per kilogram of body weight in a 4 per cent solution. Of 25 animals that died within thirty days, 14 per cent disclosed necrosis of the epithelium of the proximal convoluted tubules of the kidneys similar

8. Karsner, H. T.: Human Pathology, ed., 5, Philadelphia, J. B. Lippincott Company, 1938, p. 686.

9. Duff and Starr.<sup>2a</sup> Bailey and co-workers.<sup>2c</sup> Goldner and Gomori.<sup>5a</sup>

to that seen in mercury bichloride poisoning. In at least 8 of these, the necrosis was severe enough to account for the uremia in which the animals died.

Ten of 11 animals that died within the first four days after the injection of alloxan disclosed extensive necrosis of the peripheral portions of the hepatic lobules. Such changes have not been previously reported observed in alloxan diabetes.

In the pancreas the disappearance of necrotic islets of Langerhans may be accounted for by the traceable proliferation of adjacent exocrine cells rather than by mere absorption of the detritus and collapse of the "unsupported" walls.

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## HEART WEIGHT

### II. The Effect of Tuberculosis on Heart Weight

PEARL M. ZEEK, M.D.  
CINCINNATI

ONE of the beliefs shared by many pathologists and clinicians is that tuberculous patients are liable to have small hearts. A survey of the literature on this subject discloses four hypotheses concerning the nature of the so-called small hearts found in tuberculous patients: (1) that these are congenitally small hearts and that the resultant poor circulation in the lungs and elsewhere has predisposed to the development of tuberculosis; (2) that these hearts are really not small at all but just look that way by roentgenogram because of a change in position which produces the so-called dropped heart; (3) that small hearts in bedridden tuberculous patients may represent muscular atrophy of relative disuse; (4) that these hearts have decreased in weight because of the emaciation associated with the disease, which has similarly affected other organs and tissues, including other muscular structures.

The first hypothesis is an old one. Two of its adherents were Guarini<sup>1</sup> and Brehmer,<sup>2</sup> the latter believing that the heart of the tuberculous patient is too small for the lungs, so that there occurs a relative anemia of the lungs which predisposes to tuberculosis. Sweeney<sup>3</sup> challenged this opinion on the grounds that the more vascular tissues are the ones that are susceptible to tuberculosis, not the relatively avascular ones.

The second hypothesis is based on roentgenologic studies, which do not yet provide accurate criteria of heart weight.

The third hypothesis was suggested by White,<sup>4</sup> who said, however, that such cardiac atrophy is infrequent and in most cases slight.

The most commonly expressed hypothesis is the fourth one, but little statistical or experimental evidence has been reported to support it. In 1854 Peacock<sup>5</sup> studied the weights of healthy and diseased hearts and concluded that "the weight of the heart in phthisis was intermediate between that of cases dying of acute and other chronic disease,"

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From the Department of Pathology, University of Cincinnati, and the Cincinnati General Hospital.

1. Guarini, cited by Potthoff.<sup>14</sup>

2. Brehmer, H.: Separat-Abdruck aus den Mittheilungen aus Dr. Brehmers Heilanstalt für Lungenkranke, Weisbaden, J. F. Bergmann, 1889.

3. Sweeney, J. A.: Am. Heart J. 20:345, 1940.

4. White, P. D.: Heart Disease, New York, The Macmillan Company, 1937.

5. Peacock, T. B.: Monthly J. M. Sc. 19:193, 313 and 403, 1854.

and he pointed out that pulmonary obstruction of blood flow tends to cause cardiac enlargement but is counteracted by emaciation. It making up his series of cases with "healthy heart" he did not exclude cases of hypertension and arteriolar sclerosis; therefore the heart weights in the cases of tuberculosis in his series tended to be somewhat greater than those of the present study. In 1892 Potain<sup>6</sup> noted that the hearts of phthisic patients were unusually small and attributed this to the cachexia which accompanies the disease. He expressed the belief that if the progress of the disease was very slow, large hearts might be found, with the enlargement being due to extrapulmonary causes.

Pearl and Bacon,<sup>7</sup> in a statistical study of the records of 5,000 routine autopsies, found 1,341 in which tuberculous lesions were noted. They studied the relation of the heart weight to the weights of other viscera in tuberculous and nontuberculous persons but realized that in both groups there probably were nontuberculous lesions of the cardiovascular system which were of far greater significance in relation to the variations in heart weight than was tuberculosis. Nevertheless, they concluded that active tuberculosis tended definitely to be associated with a reduced absolute weight of the heart, compared with the heart weights of patients who had inactive tuberculous lesions. The authors stated that their study did not show whether persons with tuberculosis had small hearts because they had tuberculosis, or whether they had tuberculosis because congenitally they had relatively small hearts. In a subsequent study of these same records they found variations in the mean heart weights of different age groups, but they did not rule out hypertension and arteriolar sclerosis, and, consequently, greater than normal mean heart weights occurred in age groups in which these conditions might be expected to produce an effect on the weight of the heart. Body weights were not known, and no mention was made of body nourishment or of body length. However, it seems evident that in their series of cases active tuberculosis was associated with decreased heart weight.

Krieger<sup>8</sup> studied the atrophy of human viscera associated with inanition. In 39 cases of tuberculosis there was an average decrease in heart weight of 31.9 per cent, while the average decrease in body weight was 43 per cent. He used Grtner's formula (based on height) for normal body weight and heart weight.

Tallquist,<sup>9</sup> in a study of the small heart, listed tuberculosis as one of the associated pathologic conditions. Fishberg<sup>10</sup> stated that usually in

6. Potain, P. C., cited by Porter and Gordon.<sup>15</sup>

7. Pearl R., and Bacon, A. L.: Johns Hopkins Hosp. Rep. **21**:157 and 297, 1920.

8. Krieger, M.: Ztschr. f. ang. Anat. u. Konstitutionslehre **7**:87, 1920.

9. Tallquist, T. W., abstracted, J. A. M. A. **7**:1380, 1921.

10. Fishberg, M.: Pulmonary Tuberculosis, Philadelphia, Lea & Febiger, 1922.

tuberculosis "the heart is small, weak and atrophic, the individual muscle fibers being atrophied and fatty." Simon and Baum<sup>11</sup> studied the electrocardiograms of 250 persons with chronic pulmonary tuberculosis but found few departures from normal, and these were not striking. Anderson,<sup>12</sup> who also studied electrocardiograms, stated that "tuberculosis has no specific effect on the heart aside from that produced by any chronic, debilitating malady."

Hawes<sup>13</sup> stated that "in most instances the small heart in tuberculosis is due to the wasting effect on heart muscle, just as the leg, arm, and shoulder muscles waste away from the same cause." Pott-hoff<sup>14</sup> studied the heart shadows in the roentgenograms of 600 tuberculous men. He compared the mean transverse diameters of the heart shadows in the various body length groups with Hammer's figures for the same groups of healthy males and found that in each group the mean for tuberculous men was smaller than the corresponding mean in Hammer's series. Porter and Gordon<sup>15</sup> studied the tele-roentgenograms of 400 tuberculous patients but drew no conclusions as to the effect of tuberculosis on the size of the heart. They did not rule out hypertension and other causes of myocardial hypertrophy, and they made no mention of body nourishment except as it was indicated in body weight. Higgins<sup>16</sup> studied the relative weights of the right and left ventricles in a series of 600 autopsies of tuberculous patients and stated that the ratio of the weight of the right to that of the left ventricle is more significant than the total cardiac weight when one is looking for evidence of right ventricular hypertrophy in emaciated bodies.

#### PRESENT INVESTIGATION

In a series of 926 adults with relatively normal hearts (the cases were collected from the records of the Cincinnati General Hospital for a previous study on the weight of the normal human heart<sup>17</sup>) there were 221 in whom active tuberculous lesions were presented at autopsy. These lesions varied from active foci in lymph nodes, found only microscopically, to far advanced lesions which were the cause of death. No adult was included in this series of 221 in whom only inactive lesions were found.

In the preceding study of the normal heart three factors found to produce effects on heart weight were sex, body length and body nourish-

11. Simon, S., and Baum, F.: Am. Rev. Tuberc. **17**:159, 1928.
12. Anderson, A. R.: Am. Rev. Tuberc. **20**:728, 1929.
13. Hawes, J. B., Jr: New England J. Med. **207**:874, 1932.
14. Potthoff, F.: Beitr. z. Klin. d. Tuberk. **88**:187, 1936.
15. Porter, R. E., and Gordon, W. H.: Am. Rev. Tuberc. **36**:82, 1937.
16. Higgins, G. K.: Am. Rev. Tuberc. **49**:255, 1944.
17. Zeek, P. M.: Arch. Path. **34**:820, 1942.

ment. Table 1 presents the group distribution of the cadavers with active tuberculous lesions and the nontuberculous cadavers of the present series according to these three factors and also gives the mean heart weight for each group. The bodies classified in the preceding study as "obese" and "muscular" respectively and also those whose lengths were at the extremes of the body length distribution curves were not included in table 1 because there were too few in each category.

TABLE 1.—*Heart Weight in Tuberculous and Nontuberculous Bodies*

	Males				Females			
	Emaciated		Normally Nourished		Emaciated		Normally Nourished	
	Tu- bercu- lous	Nontu- bercu- lous	Tu- bercu- lous	Nontu- bercu- lous	Tu- bercu- lous	Nontu- bercu- lous	Tu- bercu- lous	Nontu- bercu- lous
Total number.....	73	63	55	297	60	55	23	100
Body length, 145-149 cm.								
Number of cases.....	..	..	..	..	3	3	1	14
Mean heart weight, Gm.....	....	....	....	....	208.3	230.0	210.0	248.9
Body length, 150-154 cm.								
Number of cases.....	3	3	1	5	6	10	3	40
Mean heart weight, Gm.....	228.3	258.3	265.0	299.0	198.3	209.0	226.7	246.3
Body length, 155-159 cm.								
Number of cases.....	4	5	..	11	16	12	6	47
Mean heart weight, Gm.....	240.0	230.0	....	298.2	212.5	225.4	256.7	259.7
Probable error of means, Gm.....	...	...	...	...	6.3	4.6	8.6	3.9
Body length, 160-164 cm.								
Number of cases.....	14	9	11	40	16	16	5	56
Mean heart weight, Gm.....	239.6	252.8	300.5	312.4	209.4	213.1	272.0	266.5
Probable error of means, Gm.....	6.0	6.5	8.4	6.3	4.5	6.9	12.7	3.7
Body length, 165-169 cm.								
Number of cases.....	18	19	18	66	12	11	5	24
Mean heart weight, Gm.....	252.2	265.0	296.9	304.8	218.3	230.0	274.0	274.6
Probable error of means, Gm.....	6.5	6.0	5.1	3.7	6.0	4.4	7.5	5.3
Body length, 170-174 cm.								
Number of cases.....	18	20	17	82	5	..	3	10
Mean heart weight, Gm.....	263.9	238.5	307.1	322.4	245.0	....	246.7	268.0
Probable error of means, Gm.....	5.7	7.5	4.7	2.9	....	....	....	....
Body length, 175-179 cm.								
Number of cases.....	4	3	4	58	2	3	..	8
Mean heart weight, Gm.....	308.8	285.0	322.5	330.9	282.5	258.3	....	300.6
Body length, 180-184 cm.								
Number of cases.....	8	3	2	29	..	..	..	..
Mean heart weight, Gm.....	278.1	240.0	302.5	344.5	....	....	....	....
Body length, 185-189 cm.								
Number of cases.....	4	1	2	6	..	..	..	..
Mean heart weight, Gm.....	276.3	350.0	365.0	346.7	....	....	....	....

to provide a valuable mean. Therefore the total number of bodies in table 1 is 825 instead of 926.

Within each body length group the difference in heart weight between emaciated tuberculous and emaciated nontuberculous bodies is less than that between emaciated tuberculous and normally nourished tuberculous bodies. To test the significance of these differences, the following formula was used:

$$\frac{\text{Mean}_A - \text{Mean}_B}{\sqrt{(\text{P.E.}_A)^2 + (\text{P.E.}_B)^2}}$$

This represents the difference between two mean heart weights divided by the probable error of that difference. Pearl says: "We may regard a difference of less than three times its probable error as probably not significant, one of between three and four times as probably significant, and one of more than four times as for practical purposes certainly significant."

Table 2 shows that the difference in mean heart weight between tuberculous and nontuberculous persons of similar states of body nourishment is not significant, while the difference between emaciated tuberculous and normally nourished tuberculous persons is definitely significant. Therefore, tuberculous persons can be expected to have small hearts only if they are emaciated. When they are normally nourished, their

TABLE 2.—Significance of the Difference in Mean Heart Weight Between Tuberculous and Nontuberculous Groups

	Meana — Meanb $\sqrt{(P.E.A)^2 + (P.E.B)^2}$ (Male)*			Meana — Meanb $\sqrt{(P.E.A)^2 + (P.E.B)^2}$ (Female)*		
	Body Length, Om.			Body Length, Cm.		
	160-164	165-169	170-174	155-159	160-164	165-169
When A = emaciated non-tuberculous cadavers, and B = emaciated tuberculous cadavers .....	1.2	1.5	0.6	1.7	0.5	1.7
When A = normally nourished nontuberculous cadavers, and B = normally nourished tuberculous cadavers .....	1.1	1.3	2.8	0.3	0.4	0.6
When A = normally nourished tuberculous cadavers, and B = emaciated tuberculous cadavers .....	5.9	5.4	5.8	4.2	4.6	5.8
When A = normally nourished nontuberculous cadavers, and B = emaciated nontuberculous cadavers .....	6.6	5.6	7.9	5.7	6.8	6.5

\* See text for explanation of the formula.

heart weights can be expected to be within the normal range. Table 2 shows also that within each body length group there is a significant difference between the mean heart weight of normally nourished non-tuberculous persons and that of emaciated nontuberculous persons. Therefore emaciation can be expected to be associated with decreased heart weight whether tuberculous or nontuberculous in origin.

Since the weights of the bodies in this series are not known, there is no criterion for comparing degrees of emaciation in tuberculous and nontuberculous persons. However, the departures from normal in heart weights can be compared within certain limits. Among the 926 subjects collected for these studies on heart weight there were 257 described as emaciated. Table 3 shows the distribution of these in relation to normality of heart weight. The weight of each heart was compared

with the mean heart weight for normally nourished bodies of the same sex and body length.<sup>17</sup> The weight was considered normal if it fell within the limits of the standard deviation (40 Gm. for males, and 30 Gm. for females).

In 70 per cent of the 257 cadavers showing emaciation from any cause, the heart weights were less than the lower limits of normal for bodies of similar sex and body length. Of those heart weights which were within the normal range, an additional 18 per cent (25 males and 22 females) were below the mean of their respective groups. Therefore, other factors remaining constant, hearts in emaciated bodies can be

TABLE 3.—*Heart Weight in Emaciated Bodies*

Heart Weight	Males		Females		Total	
	No.	%	No.	%	No.	%
Less than normal (mean — S. D.).....	95	70	85	70	180	70
Normal (mean $\pm$ S. D.).....	38	28	31	26	69	27
More than normal (mean + S. D.).....	3	2	5	4	8	3
	136	100	121	100	257	100

TABLE 4.—*Amount of Decrease in Heart Weight in Various Diseases Associated with Emaciation*

Grams Below Normal Heart Weight Range	Cases of Active Tuberculosis	Cases of Cancer	Cases of Addison's Disease
1-9.....	10	1	..
10-19.....	13	7	..
20-29.....	16	5	1
30-39.....	20	6	2
40-49.....	11	4	..
50-59.....	11	7	1
60-69.....	4	2	..
70-79.....	4	6	1
80-89.....	3	1	..
90-99.....	2	1	..
100-109.....	3	1	..
Total.....	97	41	5

expected to weigh less than hearts in normally nourished ones. Among the 180 emaciated bodies with heart weights below the normal range, 97 presented active lesions of tuberculosis, 41 cancer, 2 both of these conditions and 5 Addison's disease. Table 4 presents the amount of decrease in heart weight associated with these conditions. When the mean decrease in heart weight in the emaciated tuberculous bodies was compared statistically with that in the emaciated bodies with cancer no significant difference was found. However, the numbers in these groups are small, and the standard deviations are large. Also the degree of emaciation, which was not evaluated in this series, probably plays a significant role in determining the amount of

decrease in heart weight, although it has been found by Gray and Mahan<sup>18</sup> and by others that the heart weight does not diminish to the same degree as does the body weight.

In table 4 it is interesting to note how great may be the decrease in heart weight associated with emaciation. There is now in progress in this laboratory a study of small hearts, collected from autopsy material over a period of years, to determine what microscopic and clinical manifestations may be associated with decrease in cardiac weight.

#### SUMMARY

When there is emaciation, tuberculous or nontuberculous in origin, and when no cause for myocardial hypertrophy is present, heart weight can be expected to be less than normal. The decrease in heart weight is significantly associated with emaciation but not with tuberculosis as such. Similar decrease in the heart weight is found when emaciation is associated with cancer. In a tuberculous person who shows a normal state of bodily nourishment no diminution in heart weight is to be expected.

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18. Gray, H., and Mahan, E.: Am. J. Phys. Anthropol. 1:271, 1943.

# GENESIS OF AORTIC PERFORATION SECONDARY TO CARCINOMA OF THE ESOPHAGUS

Report of Observations in Two Cases

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A TERMINAL complication of carcinoma of the esophagus is rupture into one or more adjacent structures or regions. The lesion may perforate into the mediastinum, the trachea, the bronchus, the lung substance by extending directly through the pleura, or even into the aorta or other large blood vessel.

Our files contain records of 46 cases of carcinoma of the esophagus in which necropsy was done. Perforation had occurred in 22 of these, and in a few more than one structure was involved. Both the mediastinum and the lung were perforated in 1 case, the trachea and the mediastinum in 2, and in 1 instance the tumor had eroded into the aorta as well as into the trachea. The relative frequency of involvement of the structures named is as follows:

Mediastinum .....	9
Trachea or bronchus.....	10
Lung .....	5
Aorta .....	2

From this tabulation it is evident that erosion of the aorta is one of the rarer complications. It had occurred twice in the 46 cases. Carr and Hanford<sup>1</sup> have adequately summarized the statistical frequency of this form of complication by quoting the figures given by various authors.

The number of cases of perforation of the aorta due to carcinoma of the esophagus appearing in the literature is not very great. Knaut<sup>2</sup> up to 1896 had collected 34 such examples. Carr and Hanford<sup>1</sup> gathered 17 cases reported between 1896 and 1922 and added a case of their own.

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From the Department of Pathology, Medical College of the State of South Carolina.

1. Carr, J. G., and Hanford, C. W.: Am. J. M. Sc. **164**:340, 1922.

2. Knaut, B.: Ueber die durch Speiseröhrenkrebs bedingten Perforationen der benachbarten Blutbahnen, nebst einer Beobachtung von primärer Oesophagusdilatation und von Leukoplakia oesophagi, Inaugural Dissertation, Berlin, Vogt, 1896.

Since then 6 additional cases,<sup>3</sup> (including 1 case reported by Barron, which was overlooked in the aforementioned survey) have appeared. The 2 cases to be reported in the following pages bring the total to 60 recorded cases. These 2 cases are of further interest because the post-mortem observations illustrate the genesis of the perforation.

#### REPORT OF CASES

CASE 1.—C. W. J., a Negro woman 76 years of age, was admitted to Roper Hospital in March 1945 because of progressive dysphagia since October 1944. This was accompanied by recurrent attacks of sharp, stabbing substernal and precordial pain usually lasting ten to fifteen minutes and not related to ingestion of food. Occasional vomiting of food and blood-tinged material occurred, with much froth in the vomitus. For the last two to three months the stools had been very dark, almost tarry.

Examination on admission revealed marked deviation of the trachea to the right. The lungs were clear to percussion and auscultation. The heart was shifted slightly to the right of the midsternal line, and a systolic murmur was heard at the aortic and apical areas. The blood pressure was 165 systolic and 80 diastolic. The abdomen presented nothing unusual.

Blood counts gave 4,060,000 and 3,800,000 erythrocytes and 7,800 leukocytes per cubic millimeter. Urinalysis showed no abnormality on several occasions. The Kline test was positive, whereas the Wassermann reaction was negative.

While the patient was in the hospital a cough developed, continuous and persistent, with expectoration of considerable amounts of mucoid to mucopurulent sputum, sometimes blood tinged and at other times containing yellowish particulate material. On the second day after admission she began to complain of a dull aching pain in the right axilla and under the sternum. On the tenth hospital day she had a sudden massive hemorrhage from the nose and the mouth and died immediately.

*Necropsy* (eighteen hours after death).—The body was that of an emaciated Negro woman.

On the anterior surface of the esophagus, just distal to the bifurcation of the trachea, was a circumscribed, annular ulcerating neoplasm measuring 6 cm. in the greatest diameter. The edges of the lesion were elevated, rounded, gray-white and crisp, and the base consisted of discolored, gray-brown degenerating tissue. The tumor had eroded into the right main bronchus, producing a bronchoesophageal fistula measuring 8 mm. in diameter. The esophagus was densely bound down by neoplastic tissue to the aorta in this region, and here was found a sinus tract between the lumen of the esophagus and that of the aorta. This communication measured 3 mm. in diameter, and on the intimal surface of the aorta surrounding the perforation was a heaped, reddish thrombotic nodule measuring 4 mm. in all diameters.

The opening of the bronchoesophageal fistula was situated 2 cm. distal to the carina. Both bronchi and all the bronchioles were filled with clotted blood.

3. (a) Barron, M.: J. A. M. A. 67:1585, 1916. (b) Meyer, J. J.: ibid. 79: 1335, 1922 (c) Nonnis, G.: Gior. di clin. med. 9:585, 1928. (d) Polson, C. J., and McIntosh, W.: Lancet 2:960, 1931. (e) Fujishiro, Z.: Oto-rhino-laryng. 11:151, 1938. (f) Schattenberg, H. J., and Ziskind, J.: Am. J. Clin. Path. 9:615, 1939.

The blood had been aspirated even into the alveoli. In addition the lungs presented metastatic tumor nodules in the parenchyma. Tumor metastases were also found in the kidneys, in the left adrenal gland and in the periesophageal lymph nodes.

The stomach and the small intestine contained approximately 1,500 cc. of fluid and clotted blood.

Microscopically, the esophageal tumor was composed of columns of epithelial cells involving the entire thickness of the wall. Epithelial "pearls" were absent, and mitotic figures and tumor giant cells were numerous. Several acute abscesses were present in the wall of the esophagus in the region of the neoplasm.

Sections of the aorta at the edge of the perforation showed a thickened adventitia infiltrated by similar anaplastic tumor cells arranged in cords and sheets. Tumor cells were also found in perineural and perivascular lymphatics. Many thin-walled vessels, presumably the veins of the vasa vasorum, were distended and thrombosed by tumor cells. In addition a few arterioles, also in the adventitia, were thrombosed, the thrombus being composed of young fibroblasts filling the lumen. An intercostal artery, in its short course through the adventitia and near the perforation, showed tumor cell invasion of its outer coat (fig. 1 A) and organizing thrombosis of its lumen (fig. 1 B). The media of the aorta was not involved by the neoplasm, and at the edge of the perforation it was lined by acellular amorphous eosinophilic material which did not stain for fibrin or elastic tissue. A small thrombus, composed of laminated fibrin and polymorphonuclear leukocytes and laden with a mixture of bacteria, lined the intima at the edge of the perforation. Bacteria of the same type were found along the entire thickness of the rupture. The eroded edge of the bronchus was lined by tumor cells throughout its thickness.

CASE 2.—A. S., a Negro woman 38 years of age, came to the outpatient clinic of Roper Hospital on Dec. 22, 1942, complaining of sharp pain under the left breast in addition to mild dysphagia for the past four months. There had been slight nausea and vomiting on occasions. No respiratory complaints were elicited.

The patient returned to the clinic two months later and in the interim had lost 10 pounds (4.5 Kg.). Dysphagia became progressively more severe so that even water was swallowed with difficulty. She apparently refused hospitalization and three weeks later was seen in a critical condition in the Roper Hospital emergency room, where she died within a few minutes. The terminal event was marked by loss of a moderate amount of bright red blood through the nose and the mouth.

Examinations were noncontributory, and a roentgenogram of the chest when the patient was first seen revealed normal lung and heart fields.

At the time of her first clinical visit the blood showed a hemoglobin content of 11 Gm. per hundred cubic centimeters and a leukocyte count of 6,950 per cubic millimeter. The urine revealed nothing unusual.

*Necropsy* (eleven hours after death).—The body was that of an emaciated Negro woman weighing 90 pounds (41 Kg.).

The esophagus in its middle third presented an indurated, ulcerated lesion girdling the circumference of the wall and causing stenosis. This lesion measured from 3 to 6 cm. along the longitudinal axis of that organ. On the anterior aspect of the esophagus there was a defect in the tumor mass, 1 by 1.5 cm., communicating with an irregular cavity 2 by 3 by 4 cm. lying in the tissue between the aorta and the esophagus and densely adherent to the former. The

cavity contained recent and altered blood and was lined by ragged gray tissue. The periesophageal as well as the other posterior mediastinal and bronchial lymph nodes contained metastatic tumor.



Fig. 1.—*A*, portion of the wall of an intercostal artery showing clumps of tumor cells in its outer layer on the right side of the photograph. Hematoxylin and eosin;  $\times 100$ .

*B*, cross section of the intercostal artery, the lumen of which is occluded by an organizing thrombus. Weigert's elastic tissue stain;  $\times 75$ .

The intima of the aorta at the junction of the arch and the descending thoracic portion showed an indefinite, irregular grayish discoloration 1 cm. in diameter. This was situated on the anteromesial aspect of the vessel, and in its center were two minute perforations of the intima, the larger 1 by 2 mm. in diameter. These erosions extended into the aforementioned mediastinal cavity which communicated with the esophagus.

The stomach and the small intestine contained about 2,000 cc. of fluid and clotted blood. The remaining viscera showed considerable pallor and there was no evidence of metastases.

Microscopically, the esophageal tumor consisted of masses of epithelial cells arranged in sheets and cords, infiltrating the entire thickness of the wall. Numerous mitoses and tumor giant cells in addition to an occasional "pearl" were noted.

A striking feature of the aorta at the point of rupture was the tremendous thickness of the adventitia, this coat being three times the width of the media. The thickening was due to an increased amount of dense collagenous connective tissue, muscle fibers and occasional elastic fibrils in addition to clumps and cords of tumor cells. Only the adventitia was invaded by the neoplasm. The vasa vasorum showed an apparent decrease in their number and most of those that were present showed their lumens occluded by organizing thrombus material, which consisted of proliferating fibroblasts. This was observed on serial sections of the aorta taken near the edge of the perforation. Another striking feature was the distention of venules and other thin-walled vessels in the adventitia caused by tumor thrombi (fig. 2). The media and the intima at the point of perforation showed an acellular amorphous necrotic debris, which again did not take the fibrin or the elastic tissue stains. The intima surrounding the erosion presented a thrombus composed of fibrin and polymorphonuclear leukocytes.

Gram-positive and gram-negative bacilli and cocci were sprinkled through the mural thrombus on the intima and in the necrotic debris along the edge of the perforation.

#### COMMENT

Carcinoma of the esophagus begins in the mucous membrane and may infiltrate the entire thickness of the esophageal wall. Spread of the tumor may then involve, by continuity of tissues, the surrounding structures, including the aorta. The only portion of the aorta that is invaded by tumor cells is the adventitia; the media and the intima are spared. This was true in both of our cases, as shown by serial sections of the aorta at the point of perforation. This was also true in those reported cases in which the aorta at the area of rupture was described microscopically.

Indeed a noteworthy finding in both of our cases was the thrombosed vasa vasorum, the thrombi in some of the vessels being due to tumor. In one instance the lumen of an intercostal artery was filled with proliferating fibroblasts. That intercostal arteries give rise to nutrient vessels to the aorta has been demonstrated by Robertson.<sup>4</sup>

4. Robertson, H. F.: Arch. Path. 8:881, 1929.

The thrombosis of the vasa vasorum suggests the genesis of the aortic perforation. Vierhuff<sup>5</sup> in 1896 recognized that impairment of the blood supply of the aorta was an important factor leading to its rupture. He observed that only the adventitia of the aorta was invaded by tumor and that the media at the point of perforation was composed of necrotic material due to "defective tissue nourishment from destruction of the vasa vasorum."

According to Schattenberg and Ziskind,<sup>3f</sup> the perforation occurs usually as the tumor "disintegrates before the advancing ulcerative process" and in some cases because of suppuration of the tumor. The latter part of their statement obviously suggests perforation due to bacterial invasion of the tumor. This view was upheld by Barron,<sup>3a</sup> who demonstrated bacteria in the perforation and only a few tumor

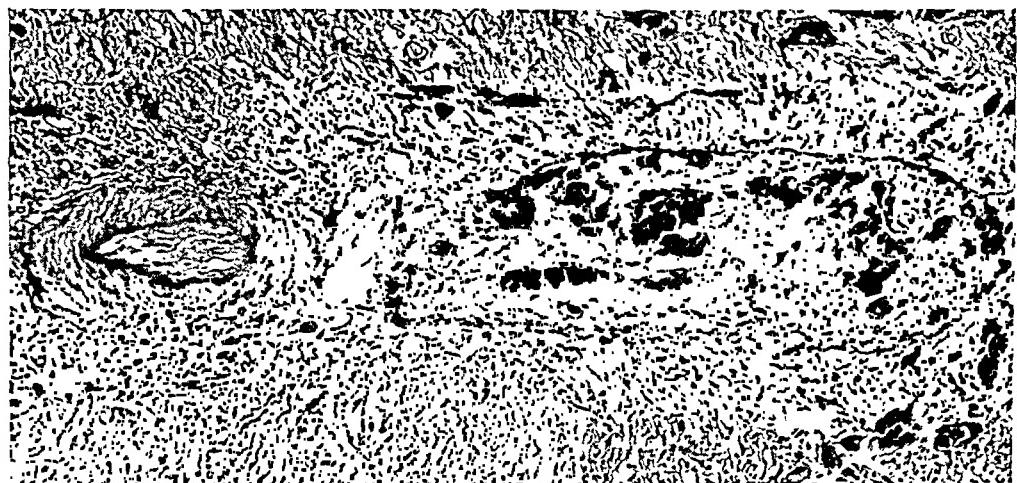


Fig. 2.—Section through the adventitia of the aorta in case 2. An arteriole occluded by proliferating fibroblasts is shown on the left. To the right is a thin-walled vessel distended with tumor cells;  $\times 100$ .

cells in the adventitia while none were present in the media and the intima. He therefore concluded, since the carcinomatous invasion of the aorta was minimal and the amount of infection was profound, that the latter contributed greatly to the perforation.

It seems that of the authors mentioned only Vierhuff has considered the condition of the vasa vasorum in this disease process. Obviously, the aorta depends for its nourishment on the integrity of these vessels, and any disease that involves them will be ultimately reflected on that great vessel. Since there was a conspicuous decrease in the number of the vasa vasorum in the thickened adventitia and since most of those that were present were thrombosed either by

5. Vierhuff, W.: St. Petersburg med. Wchnschr. 13:195, 1896.

proliferating fibroblasts or by invading neoplasm, we feel that the perforation in both cases was due to interference with the blood supply. Furthermore, we are of the opinion that examination of the aorta at the site of perforation will reveal thrombosed vasa vasorum in all cases.

Although bacteria were present in our sections of the aorta, they were most likely saprophytes or even postmortem invaders and not true pathogens. This is substantiated by the fact that amorphous material was present at the perforation which did not contain fibrin and further by the fact that the site showed no inflammatory cellular reaction.

#### CONCLUSIONS

Perforation of the aorta due to carcinoma of the esophagus is of infrequent occurrence. Study of 2 cases indicated that perforation of the aorta results from interference with the blood supply secondary to thrombosis of the vasa vasorum and not from tumor invading the entire vessel wall nor from bacterial infection.

# Case Reports

## ENTERITIS CAUSED BY SALMONELLA SUIPESTIFER WITH SECONDARY MONILIASIS

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IT IS the purpose of this paper to describe the results of a pathologic examination of a patient succumbing to *Salmonella suipestifer* infection complicated by moniliasis of the upper gastrointestinal tract. The latter was unsuspected during life.

Longcope<sup>1</sup> was the first to describe the clinical features of *S. suipestifer* infection. In 1902 he reported 2 cases. One patient came to autopsy; the other recovered. No ulcers were seen in the intestines, and only a few focal necroses were present in the liver. In 1918 Neukirch<sup>2</sup> reported a study of patients many of whom presented symptoms of typhoid. He cultivated an organism, which he called *Bacillus erzindjahn*, and which probably belonged to the paratyphoid group B. One of the patients, whose illness resembled most closely an attack of dysentery, showed at autopsy irregular fresh ulcers of the colon. Neukirch drew a parallel between *S. suipestifer* and *B. erzindjahn* as far as virulence and invasiveness was concerned. In 1937 Harvey<sup>3</sup> summarized the literature and added a report of new cases. The diseases caused by the *Salmonella* group were discussed by Bornstein<sup>4</sup> in 1943. Harvey noted the conspicuous absence of intestinal lesions in *S. suipestifer* infection and at the same time stated that "in only an occasional case" is the organism cultivated from the stool. The postmortem findings, according to Bornstein, are not characteristic. Seligmann, Saphra and Wassermann,<sup>5</sup> who analyzed 1,000 cases of *Salmonella* infection collected at the New York *Salmonella* Center, divided the clinical manifestations into (a) predominantly gastroenteric signs, (b) localized processes due to contact or hematogenous spread and (c) typhoid-like symptoms and symptoms of septicemia without localizing signs. For the sake of completeness, the asymptomatic carrier state was included. The case at hand combines gastroenteric symptoms with those of septicemia.

### REPORT OF A CASE

E. F., a 60 year old white married woman, a dressmaker, was admitted to the medical service of the Peter Bent Brigham Hospital for the first time June 9,

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From the Department of Pathology, Peter Bent Brigham Hospital, and the Department of Pathology, Harvard Medical School.

1. Longcope, W. T.: Am. J. M. Sc. **124**:209, 1902.

2. Neukirch, P.: Ztschr. f. Hyg. u. Infektionskr. **85**:103, 1918.

3. Harvey, A. M.: Arch. Int. Med. **59**:118, 1937.

4. Bornstein, S.: J. Immunol. **46**:439, 1943.

5. Seligmann, E.; Saphra, I., and Wassermann, M.: Am. J. Hyg. **38**:226, 1943.

1944. Three days before admission she vomited violently, experienced nausea and began to have severe diarrhea. These symptoms persisted until she entered the hospital. No blood was noted in the yellow vomitus or the stools. Her temperature was reported to have been as high as 104 F.

No history of having used well water or infected milk was elicited. However, many friends at her place of work had recently had diarrhea. The past history was noncontributory.

On admission she appeared a moderately ill, slightly obese woman. Her temperature was 100 F.; the pulse rate, 80; the respiratory rate, 20, and the blood pressure, 115 systolic and 90 diastolic. The abdomen was distended and tympanitic. No fluid wave was obtained. The spleen was not felt. The examination disclosed no other significant abnormalities. A rash was not observed.

The significant laboratory findings included that of *S. suipestifer* in a stool culture on admission. On the fifth hospital day *S. suipestifer* was grown from the urine. The following day *S. suipestifer* was grown from the blood. At first the urine and blood cultures had failed to yield *S. suipestifer*. Other laboratory data of importance were: a leukocyte count of 5,250 to 5,500 per cubic millimeter, with a differential count of 52 per cent neutrophils, 14 per cent band forms, 14 per cent lymphocytes and 20 per cent monocytes on the day of admission. The erythrocyte count was 4,890,000 and the hematocrit value 44 per cent. The urine contained hyaline and granular casts, protein (2+), also acetone (1+) and sugar (1+). A guaiac test of the stools was positive. Amebas were not found.

Treatment consisted primarily of parenteral administration of fluid. The temperature ranged from 99 to 101 F., with a high of 102 F. on the third hospital day. The pulse rate also remained elevated, rising as high as 120. Thirty-six hours before death, the patient rapidly became comatose, and pulmonary congestion developed. She died on the eighth hospital day.

It should be recorded that culture of a stool from the patient's husband yielded *S. suipestifer*.

*Autopsy* (three hours after death).—The body was that of a well developed, obese, elderly white woman. The skin was white and turgid. No lesions of the skin were discovered. The peritoneum, both parietal and visceral, was smooth, grayish pink and glistening. No free fluid was encountered, and there was no exudate. There was only slight congestion of the serosal vessels of the descending colon. The stomach was distended with fluid. The mesenteric lymph nodes were not enlarged.

One hundred cubic centimeters of clear brownish fluid was present in both the right and the left pleural cavity. The heart weighed 270 Gm. and presented no gross abnormalities. The right lung weighed 560 Gm. and the left 390 Gm. The lungs were moderately voluminous, and the pleural surfaces were glistening. On section the lower lobes, particularly that on the right, appeared congested and somewhat edematous. The spleen weighed 100 Gm. It was of normal consistency. The pulp was dark reddish purple. The malpighian corpuscles were indistinct.

The most important findings were those in the alimentary tract. The esophagus was freed with difficulty from the mediastinal tissues. The lumen of the esophagus was narrowed and filled with cloudy reddish gray material. The mucosal surface appeared granular and bright red to reddish black. The discoloration ended abruptly at the gastroesophageal junction. The normal mucosal pattern of the stomach was well preserved. A 3 mm. wide triangular bright red ulcer,

which appeared to have burrowed through the mucosa, was located along the lesser curvature. Approximately twenty minute irregular similar ulcers were observed in the pyloric antrum. The mucosa in the first portion of the duodenum resembled that of the esophagus. The mucosa of the remainder of the small intestine was edematous and dusky pink to pale pink. Ulcers were not seen. Peyer's patches could not be identified.

Conspicuous shallow ulcers were visible in the dependent portion of the cecum and in the ascending and the transverse colon. Some were confluent, but the majority were discrete. They were oval or somewhat irregular in shape. Occasionally they were circinate. They measured 0.5 to 3 cm. in length and 0.3 to 1 cm. in width. The arrangement in the main was parallel with the long axis of the colon. The craters were pale yellow to yellowish brown, and they were not covered with purulent exudate. The surrounding mucosa was occasionally edematous and yellow to brownish red.

The liver weighed 1,600 Gm. and was of fairly firm consistency. The surface was pale brown to brownish yellow, and the smooth sectioned surfaces were pale brown. No abscesses were encountered in many sections.

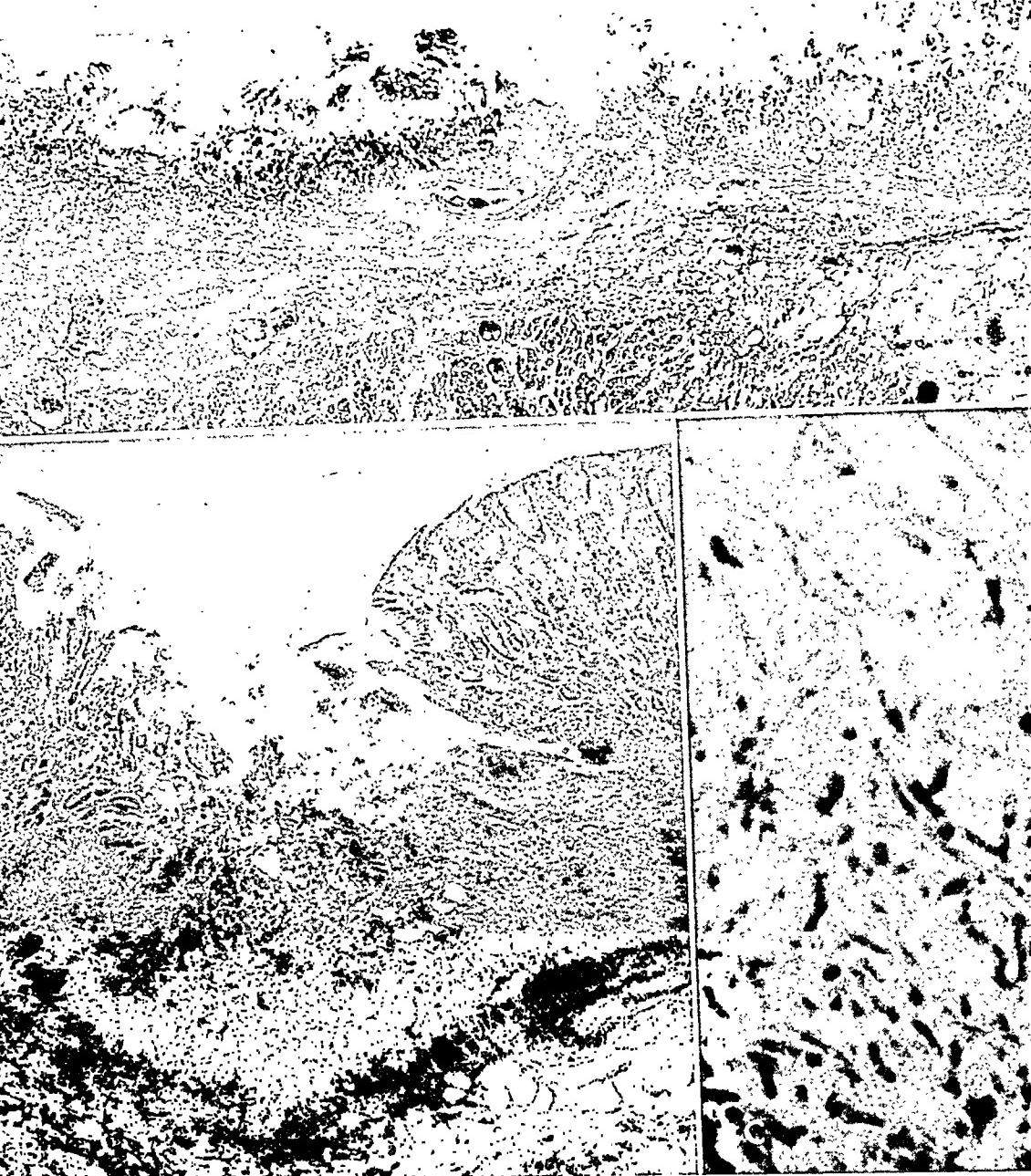
The kidneys, the adrenal glands, the bladder and the pelvic organs presented no unusual features. Tissue blocks were fixed in Zenker's solution and in Zenker's solution to which solution of formaldehyde U. S. P. had been added in the concentration of 5 per cent, instead of acetic acid. Sections were stained by the eosin-methylene blue and Gram methods.

The microscopic features of particular interest were observed in the gastrointestinal tract. The esophagus showed both mucosa and submucosa destroyed, with only fragmented strands of brown or blue necrotic material remaining. A few necrotic esophageal glands were still recognizable. It was in this necrotic material that a large number of organisms resembling moniliias were found. These were gram-positive rods ranging in length from 18 to 20 microns approximately. Mycelial strands and spores were seen. A few poorly preserved blood vessels were invaded by the moniliias. There was little or no cellular response around the fungous elements. The connective tissue between the muscle bundles was edematous and displayed markedly congested blood vessels. This loose stroma exhibited a scattered, primarily monocytic infiltration with only rare polymorphonuclear leukocytes. Occasional fibroblastic proliferation was recognized. The muscularis itself was not necrotic. Fibrin networks had become deposited in the adventitia of the esophagus, particularly near the gastroesophageal junction. Here, occasional moniliias were seen. Only little monocytic infiltration was observed in the fibrin network. The destruction of mucosa terminated abruptly at the gastroesophageal junction (*A* in figure).

In the stomach the ulcers observed grossly proved to be acute and deep, extending down into the lamina propria (*B* in figure). The margins were slightly overhung by the intact gastric mucosa. The bases of the ulcers were composed of a thick layer of mycelial threads with accompanying spores (*C* in figure). Several blood vessels at the bases of the ulcers contained thrombi which were invaded by the moniliias. Again, leukocytic response surrounding the ulcers was scant and predominantly monocytic. The muscularis was edematous. Sections taken from the gastroduodenal junction showed a process essentially similar to that shown by the esophagus, though somewhat more pronounced.

The small intestine displayed diffuse, though moderate, monocytic, lymphocytic and plasma cell infiltration, occasionally with eosinophilic polymorphonuclear leukocytes in the edematous lamina propria. The mucosa was intact, and monilial

elements were not observed. In the colon large acute ulcers were found. These were shallow, barely penetrating the lamina propria. They were sharply limited on either side by intact mucosa, which was not undermined. The bases of the ulcers were generally covered by a thin layer of fibrin enmeshing gram-negative bacilli. In many zones closely packed monocytes were seen. Only occasionally were polymorphonuclear leukocytes seen infiltrating just beneath the bases of the



A, ulceration and edema of the esophageal mucosa at the cardiac orifice. Eosin and methylene blue;  $\times 26$ . B, acute ulceration of gastric mucosa. Eosin and methylene blue;  $\times 37$ . C, monilial elements at the base of the ulcer shown in B. Eosin and methylene blue;  $\times 900$ .

ulcers. Several vessels and lymphatics were completely or partially filled with thrombi. One vessel situated at the base of one of the ulcers exhibited early thrombus formation. Some lymphatics contained polymorphonuclear leukocytes. The leukocytic response, mostly confined to the superficial zones of the strikingly edematous lamina propria, was primarily one of large monocytes with pale cytoplasm. The mucosa and the muscularis were compressed. A few vessels in the lamina propria showed fibrinoid degeneration. There was no abnormal degree of leukocytic infiltration in the intact mucosa.

The heart showed a few foci of lymphocytes beneath the pericardium. The lungs exhibited a patchy acute exudate, most pronounced and occasionally confluent around some of the bronchioles. The inflammatory cells were for the most part neutrophilic polymorphonuclear leukocytes. Almost invariably the exudate was centered around prominent colonies of gram-positive cocci. Occasional cocci were engulfed by monocytes. In addition, strands of moniliias and spores were seen in the alveoli, generally in conjunction with the cocci. Moniliias were not seen intracellularly. The lumens of the bronchioles contained similar exudate and micro-organisms. The cellular masses within the bronchioles in many instances were embedded in hyaline amorphous aggregates, which were interpreted as representing aspirated material. The alveolar capillaries were considerably congested, and there was evidence of edema in the alveolar spaces. The bronchiolar walls were infiltrated by monocytes and polymorphonuclear leukocytes.

The pulp of the spleen was markedly congested. There was only little lymphoid depletion. No bacteria were seen. The liver cells showed considerable fatty vacuolation, particularly at the periphery of the lobules. The Kupffer cells in the sinusoids were prominent. Neither bacteria nor moniliial elements were seen. In the kidney, a few hyaline casts were present in the collecting tubules, and several foci of monocytes and lymphocytes were encountered in the pelvic fat. The renal parenchyma was otherwise not remarkable. Again, bacteria could not be identified. The adrenal gland exhibited considerable lipid depletion. In the medulla minimal round cell infiltration was detected. The remainder of the sections, taken from the pancreas, the left ureter, the bladder, the uterus and the ovary, showed no significant changes.

*S. suis* was isolated from the ileum, the colon and the blood in the heart post mortem. The H antigen of organisms obtained from the heart's blood and the colon was destroyed by heating at 100 C. for fifteen minutes. The O antigen was tested against *S. suis* and *Salmonella aertrycke* stock serums. Agglutination was positive in a dilution of 1:80 against *S. suis* serum and questionably positive against *S. aertrycke* serum in a dilution of 1:10.

#### COMMENT

The lesions in the colon, which can be presumed to be the result of *S. suis* infection of the gastrointestinal tract, are probably of secondary importance in this case, since one is dealing with a blood stream infection. *S. suis* is an exceedingly invasive organism. In Seligmann's<sup>5</sup> series the blood cultures in 60 per cent of the cases of *S. suis* infection were positive. The organism is prone to invade other organs, as is emphasized by many different reports, only a few of

which are cited: involvement of the lungs,<sup>6</sup> the endocardium<sup>7</sup> and the meninges.<sup>8</sup> There may be involvement of the genitourinary tract, such as perinephric abscess,<sup>9</sup> and even such lesions as subdiaphragmatic abscess<sup>10</sup> and salpingitis.<sup>11</sup> For further reports see Seligmann and co-workers.<sup>5</sup> It should be remembered, however, that *S. suipestifer* infection is characterized by bactemia, thus bearing a close resemblance to typhoid. The site of invasion appears to be the gastrointestinal tract. Chronically infected human carriers of *Salmonella* have been reported.<sup>4</sup>

The colonic ulcers in this case had the appearance of relatively mild lesions. The lamina propria was barely penetrated, and the mucosa between the ulcers was intact. Conspicuous, however, was the marked edema of the submucosa, which was found to a lesser degree in the small intestine. Other noteworthy features were the predominantly monocytic and lymphocytic infiltration at the bases of the ulcers and the scant amount of fibrin deposition. The question might well be raised at this stage: Does *S. suipestifer* behave like *Eberthella typhosa*, and does it exercise a so-called negative chemotaxis as was shown to be the case for antigen prepared from *E. typhosa* by Morgan and Upham<sup>11</sup>? The low white cell count in this case with only 52 per cent neutrophils lends plausibility to this speculation, as does the statement by Harvey<sup>3</sup> that unless localization of the infectious process should occur, the white cell count remains at a low level. Similarly, the white cell count in the cases studied by Jager and Lamb<sup>9</sup> generally ranged between 5,000 and 10,000.

*S. suipestifer* falls into group C of paratyphoid bacilli, according to the classification of the New York *Salmonella* Center.<sup>5</sup> Gastrointestinal symptoms and lesions appear to be preeminently caused by *Salmonella typhi murium*, belonging to group B.<sup>3</sup> Angrist, in discussing a report by Bornstein,<sup>12</sup> described lesions somewhat resembling typhoid in a 21 year old patient who entered the hospital with signs and symptoms of appendicitis and later succumbed to peritonitis. The white cell count, however, was only 2,100. *S. typhi murium* was recovered from the stool and from a colonic ulcer. Peyer's patches were somewhat superficially ulcerated, hemorrhagic and packed with large mononuclear cells. Numerous small ulcers and an occasional large ulcer were found in the colon. Ulcers caused by paratyphoid A and B organisms are illustrated in an early report by Dawson and Whittington.<sup>13</sup> They found the lower 2 feet (61 cm.) of the ileum constantly affected with deep punched-out ulcers and swelling of the lymphoid follicles. The large intestine was severely involved in many instances.

6. Shaw, F. W.: J. Lab. & Clin. Med. **12**:141, 1926. Cole, D. B., and Nalls, W. L.: ibid. **23**:1223, 1938.

7. Goulder, N. E.; Kingsland, M. F., and Janeway, C. A.: New England J. Med. **226**:127, 1942.

8. Neter, E. R.: Arch. Int. Med. **73**:425, 1944.

9. Jager, B. V., and Lamb, M. E.: New England J. Med. **228**:299, 1943.

10. Hunter, J. E.; Andersen, C. A., and Hutchinson, W. E.: Northwest Med. **43**:114, 1941.

11. Morgan, H. R., and Upham, H. C.: Proc. Soc. Exper. Biol. & Med. **48**:114, 1941.

12. Bornstein, S.: New York State J. Med. **42**:163, 1942.

13. Dawson, E., and Whittington, T. H.: Quart. J. Med. **9**:98, 1915-1916.

Morphologically, there is little to suggest an overwhelming toxemia in the present case. The Kupffer cells, it is true, were somewhat prominent. However, the spleen, unlike that affected by typhoid, which is characteristically enlarged and soft, failed to show anything resembling a "splenic tumor." Therefore, Bornstein's<sup>4</sup> previous statement with regard to absence or nonspecificity of lesions in *Salmonella* septicemia is not refuted by the histologic observations in this case.

The importance of the colonic ulcers is overshadowed by the severe esophageal ulcers, the necrotizing lesions in the duodenum and the pinpoint ulcers in the stomach. Fungi having the morphologic characteristics of *Candida albicans* or moniliias were present in large numbers. Mycelial threads were lying within the necrotic esophageal and duodenal mucosa, surrounded by spores. In the stomach the organisms formed a thick layer at the bases of the ulcers. Thrombus formation within the vessels at the ulcer base was a noteworthy finding. The leukocytic response to the moniliias was primarily monocytic and lymphocytic, with slight fibroblastic proliferation. This was similar to the cellular response in the ulcers of the colon. Thus the tissue reaction appeared to be of some standing but was without resemblance to a granulomatous process. The presence of moniliias in the lung, together with numerous gram-positive cocci and an acute inflammatory reaction largely around and also to some extent within bronchioles, was consistent with aspiration pneumonia.

*C. albicans* proliferates in the tissues as mycelial threads, accompanied by budding or nonbudding ovoid forms. According to Swartz,<sup>14</sup> six species can be identified in freshly isolated cultures. Castellani<sup>15</sup> attempted differentiation by biochemical methods. The virulent strains cause death of rabbits a few days after intravenous injection. The manifestations of moniliasis are manifold; one of the most common is the oral thrush of childhood. Benham and Hopkins<sup>16</sup> recovered *C. albicans* from the alimentary tracts of 18 of 100 apparently normal young adults. Langenbeck first described *C. albicans* as a human pathogen in 1839. He found the fungus in patches of thrush on the oral mucosa, the pharynx and throughout the intestinal tract in a patient who had died of typhoid.<sup>11</sup> This early observation has a definite bearing on the pathogenic properties of moniliias. *C. albicans* is prone to attack debilitated persons or to be a secondary invader. Lewis and Hopper<sup>17</sup> stated that "there is more or less corresponding increase with age in the involvement of the gastrointestinal tract" without further amplification. Yet the condition seems to be an uncommon finding, or it is possibly overlooked at autopsy.

In a series of 5,059 consecutive autopsies from 1913 to 1943 at the Peter Bent Brigham Hospital only 3, according to the records, revealed esophageal thrush. It is noteworthy that all 3 patients were suffering

14. Swartz, J. H.: Elements of Medical Mycology, ed. 1, New York, Grune & Stratton, Inc., 1943, pp. 45-62.

15. Castellani, A.: Fungi and Fungous Diseases. Chicago, American Medical Association, 1928, p. 24.

16. Benham, R. W., and Hopkins, A.: Arch. Dermat. & Syph. 28:532, 1933.

17. Lewis, G. M., and Hopper, M. E.: An Introduction to Medical Mycology, ed. 1, Chicago, The Year Book Publishers, Inc., 1939, p. 129.

from debilitating diseases: Two had diabetes; one of these had pulmonary tuberculosis, and the other had marked arteriosclerosis with gangrene. The third patient had ulcerative colitis. Severe necrosis of the esophageal mucosa was present in 2 of the patients, while in the third the lesions were less pronounced. Polymorphonuclear leukocytes were more numerous in these lesions than in those found in the present case, although monocytes were also abundant. The case presented here bears a striking resemblance to 1 of the cases described by Irish.<sup>18</sup>

In children, on the other hand, thrush esophagitis is not an uncommon clinical entity. Ebbs<sup>19</sup> reported 28 cases of esophagitis in infancy; 22 proved to be due to *C. albicans*. Reye<sup>20</sup> described 5 cases of esophagitis due to *Monilia albicans* encountered in 150 autopsies performed on infants. The pathologic observations appear to be similar to those in the present case.

Several further points of interest are to be noted in the history. 1. The patient died in June. *Salmonella* infection is most common in the hottest months of the year, with a peak in September.<sup>5</sup> 2. There was a history of vague gastrointestinal disturbances of companion workers; *S. suipestifer* was grown from the stool of the patient's husband. Although *S. suipestifer* infections generally sporadic, there appears to be some epidemiologic background in this instance. However, no proof is available, and the hypothesis of cross infection must remain mere speculation. 3. Mortality of adults due to *S. suipestifer* infection is high.<sup>21</sup>

#### SUMMARY

A 60 year old woman was afflicted with a severe gastrointestinal disturbance and finally succumbed to the septicemia of *S. suipestifer*. The colonic ulcers probably cannot be considered responsible for the death of the patient. The severe esophagitis, the gastric ulceration and the duodenitis caused by *C. albicans* infection, shown on pathologic study, were unsuspected during life. In this instance the fungus is considered a secondary invader in a debilitated patient.

18. Irish, R. H.: New York State J. Med. **36**:1491, 1936.

19. Ebbs, J. H.: Arch. Dis. Childhood **13**:211, 1938.

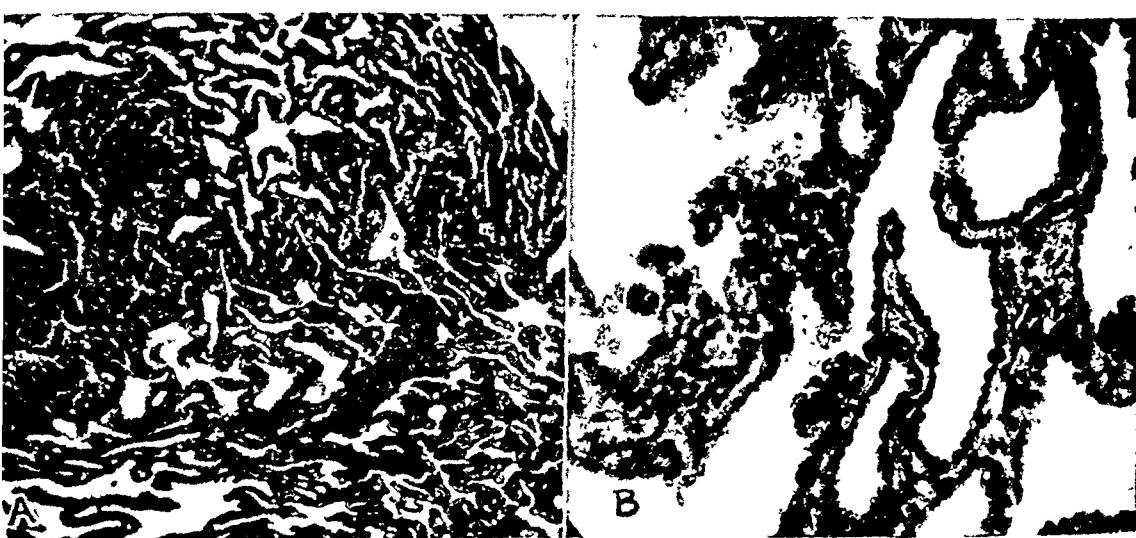
20. Reye, D.: M. J. Australia **2**:673, 1941.

21. Keefer, C. S.: New England J. Med. **222**:105, 1940.

## PRIMARY CARCINOMA OF THE LIVER OF A DOG

WALTER M. BOOKER, Ph.D., and A. C. WEBB, M.D., WASHINGTON, D. C.

IN SPITE of the large volume of work on cancers of animals, both those experimentally produced and those incidentally observed, primary carcinoma of the liver is rare in dogs. It is well known that the incidence of primary carcinoma of the liver is low also in the human race of this country and of Europe. Goodpasture<sup>1</sup> has shown that in dogs there is some relation between the incidence of tumors and senescence. Even so, the incidence of primary carcinoma of the liver in the senile animals studied by Goodpasture was low. Carcinoma of



A, section of carcinoma of the liver under low power magnification, showing the proliferating epithelium forming a marked papillary pattern and pseudoacinous production.

B, section of carcinoma of the liver under high power, showing the tumor composed of proliferating bile duct epithelium with a supporting stroma of connective tissue. In the upper half are masses of hyperchromatic cells in which the papillary pattern is lost.

the liver (not experimentally produced) has been reported observed in rats and fowl.<sup>2</sup>

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From the departments of pharmacology and pathology, Howard University School of Medicine.

1. Goodpasture, E. W.: J. Cancer Research 1:363, 1916.

2. Kahlau, G.: Frankfurt. Ztschr. f. Path. 50:361, 1937. Itami, S.: J. Cancer Research 3:275, 1918.

While making glycogen determinations on the livers of dogs in connection with another problem, the carcinoma herein reported was seen, and in view of its rarity we regarded it as being worthy of reporting. We make no claim that this is an initial contribution.

#### REPORT OF A CASE

An old female dog, weighing 15.5 Kg., somewhat decrepit and having cataracts, had been under prolonged pentothal sodium anesthesia the day before it was killed. When the liver was removed, a large mass was observed on the dorsal aspect of the large right lobe. Careful examination failed to show a tumor in any other organ or tissues.

The liver and the gallbladder together weighed 550 Gm. The external and the cut surfaces were reddish brown, and the consistency was firm. Protruding from the dorsal surface of the large right lobe of the liver there was a soft, whitish ovoid tumor that had a greater diameter of 3 cm. and a lesser diameter of 3.4 cm. It was circumscribed but not encapsulated.

Microscopically the tumor was composed of glandular tubules, varying in size and in shape, supported by a stroma of connective tissue, forming a papillary pattern. The acini were lined with a single layer of cuboidal cells with deeply staining nuclei. In some fields, however, the epithelium was thickened by masses of anaplastic cells that projected into the lumen. Mitotic figures were not frequent. There was no sharp line of demarcation between the tumor and the hepatic tissue. Some isolated glands, surrounded by hepatic parenchyma, were seen at variable distances from the main tumor. The liver cells did not show significant histologic changes (figure).

#### COMMENT

In a recent paper Mulligan<sup>3</sup> called attention not only to the low incidence of primary carcinoma of the canine liver but to the small number of reports that have been made on canine neoplasms. He has made a plea to veterinary hospitals for more routine autopsies and reasonably detailed reports of them. We believe that the plea should be extended to laboratory workers, who should at least grossly examine the organs of dead experimental animals to determine whether or not tumors are present. If this is done on a wide scale, we believe the statistics on tumors occurring in dogs and other animals will be more extensive.

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3. Mulligan, R. M.: Arch. Path. 38:115, 1944.

## HEMOPERICARDIUM FROM PERICARDIAL METASTATIC CARCINOMA

GEORGE J. RUKSTINAT, M.D., CHICAGO

DEATH from bleeding into the pericardial sac may be sudden and unexpected. Most frequently this occurrence is associated with a tear of the aorta within the pericardial sac or with cardiac rupture in a patient suffering from myocardial damage. Blood in quantities sufficient to tint the pericardial exudate is occasionally present in pericarditis associated with pneumonia and uremia. Bleeding into the pericardial sac from metastatic tumor growths in amounts capable of causing cardiac constriction and death is unusual. A case history recording such an event follows.

### REPORT OF A CASE

A painter 45 years of age, who had been a champion soccer player, entered the Loretto Hospital, June 10, 1945, for treatment of typical lobar pneumonia of the left lung and pleurisy. The presence of these conditions was confirmed by roentgen studies, which also revealed marked fibrosis throughout the right lung. The patient was given a series of intramuscular injections of penicillin (350,000 units) and made a satisfactory recovery.

Within two weeks, however, he was again stricken with pneumonia and was treated as in the first attack. Thereafter he had difficulty in breathing and his respiration was noisy.

On July 20 he again became ill and had foul sputum containing a few fusiform bacilli, a few Vincent's spirochetes and many staphylococci. Moist rales were heard in both lungs, and the liver was enlarged and tender. It was thought the fingers showed slight clubbing, and a diagnosis of cor pulmonal was made. The patient was treated with digalen. He had considerable difficulty in breathing and complained of substernal pain. During the two days of his terminal hospital stay, cardiac and respiratory stimulants failed to relieve the thoracic pain although they increased the blood pressure. On one occasion the attending physician thought that the patient was made worse by such therapy. The patient died about an hour later.

At autopsy the body was powerfully developed, was 6 feet tall and weighed 220 pounds (100 Kg.). The left pleural cavity contained 500 cc. of clear yellow liquid, and the left lung was involved in atelectasis. The right pleural cavity was obliterated by fibrous tissue, and the right lung revealed primary bronchiogenic carcinoma of the bronchus to the upper lobe. The tumor extended along the bronchus about 22 mm. and encircled about 75 per cent of the circumference. It obstructed about one third of the lumen and was firm and yellow-gray. There was extensive involvement of the surrounding parenchyma in the form of clusters of metastases, and similar growths occurred in the tracheobronchial lymph glands.

The pericardial sac contained about 160 cc. of liquid blood and about 45 Gm. of soft red-brown clot. The latter was in part attached to the epicardium, but much of it was in an irregularly ridged mass in the sac. The source of bleeding was an irregular clump of carcinomatous nodules on the epicardium close to the interventricular septum. The individual masses were 2 to 11 mm. in

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From the Department of Pathology, Loretto Hospital.

diameter, and two had oozing zones of hemorrhage as much as 0.5 mm. in diameter. The heart weighed 480 Gm. and had a normal arrangement of valve leaflets, chambers and vascular trunks.

The patient's father died of carcinoma of the stomach at 47 years of age, while his mother, aged 70 years, was suffering from carcinoma of the uterine cervix. A brother died when 26 years old, of pulmonary tuberculosis.

#### SUMMARY

In a case of fatal bleeding into the pericardial sac from epicardial carcinomatous metastases, the primary tumor, an epidermoid type of bronchiogenic carcinoma, was in the upper lobe of the right lung. The metastases reduplicated the original growth and were highly vascular. Several had bleeding regions, the blood from which, accumulating in the pericardial sac, caused cardiac compression and death.

## TERATOMA OF PINEAL GLAND WITH CHORIOCARCINOMA AND RHABDOMYOSARCOMA

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**I**N THIS report we shall record the clinical and pathologic observations made in a case of pineal teratoma in which choriocarcinoma and rhabdomyosarcoma were found. Askanazy,<sup>1</sup> van den Berg and co-workers<sup>2</sup> and Russell<sup>3</sup> have reported similar cases. Bochner and Scharff<sup>4</sup> analyzed the recorded cases and added 3 of their own in 1938, and they discussed the various types of tissue found. Additional cases were reported by Lichtenstein,<sup>5</sup> Gerstley<sup>6</sup> and Russell.<sup>3</sup> Throughout the literature on the tumor generally designated as "pinealoma" runs a controversy as to whether it is a tumor of the pineal cells or a teratoma. In the most recent article Russell concluded that in most of the instances the tumor was teratomatous, but he expressed the belief that in an occasional one the tumor may be true pinealoma. The resemblance of the tumor known as "pinealoma" to "seminoma" and "embryonal carcinoma" of the testicle and to "dysgerminoma" of the ovary is quite suggestive of a relationship.<sup>7</sup>

The desirability of investigations of the urinary gonadotropic hormone in patients with symptoms of pineal tumor has been suggested.<sup>4</sup> This case further emphasizes this possibility.

### REPORT OF A CASE

A 17 year old youth was admitted to the Indiana University Medical Center July 28, 1936. He complained chiefly of headache, vomiting, turning inward of each eye, and staggering on walking. His present illness had commenced about four weeks before admission with involuntary elevation of the eyes and headache. He then began to stagger a little on walking. Four days before coming to the hospital his eyes "turned in." At this time projectile vomiting developed. His previous medical history was essentially irrelative. He had always been thought to be quite retarded mentally.

Neurologic examination showed him to be in a semistuporous state. The skull was normal. The pupils were dilated and equal, and did not react to light. There was a marked convergence spasm of the eyes. He was unable to elevate or depress them.

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From the Departments of Neurosurgery and Clinical Pathology, Indiana University School of Medicine.

1. Askanazy, M.: Verhandl. d. deutsch. path. Gesellsch., 1906, p. 58.
2. Hijmans; van den Berg, A. A., and van Hasselt, J. A.: Nederl. tijdschr. v. geneesk. **1**:1271, 1913.
3. Russell, D.: J. Path. & Bact. **56**:145, 1944.
4. Bochner, S. J., and Scharff, J. E.: Arch. Surg. **36**:303, 1938.
5. Lichtenstein, B. W.: Arch. Neurol. & Psychiat. **44**:153, 1940.
6. Gerstley, J. R.: J. Pediat. **17**:512, 1940.
7. Harris, W., and Cairns, H.: Lancet **1**:3, 1932.

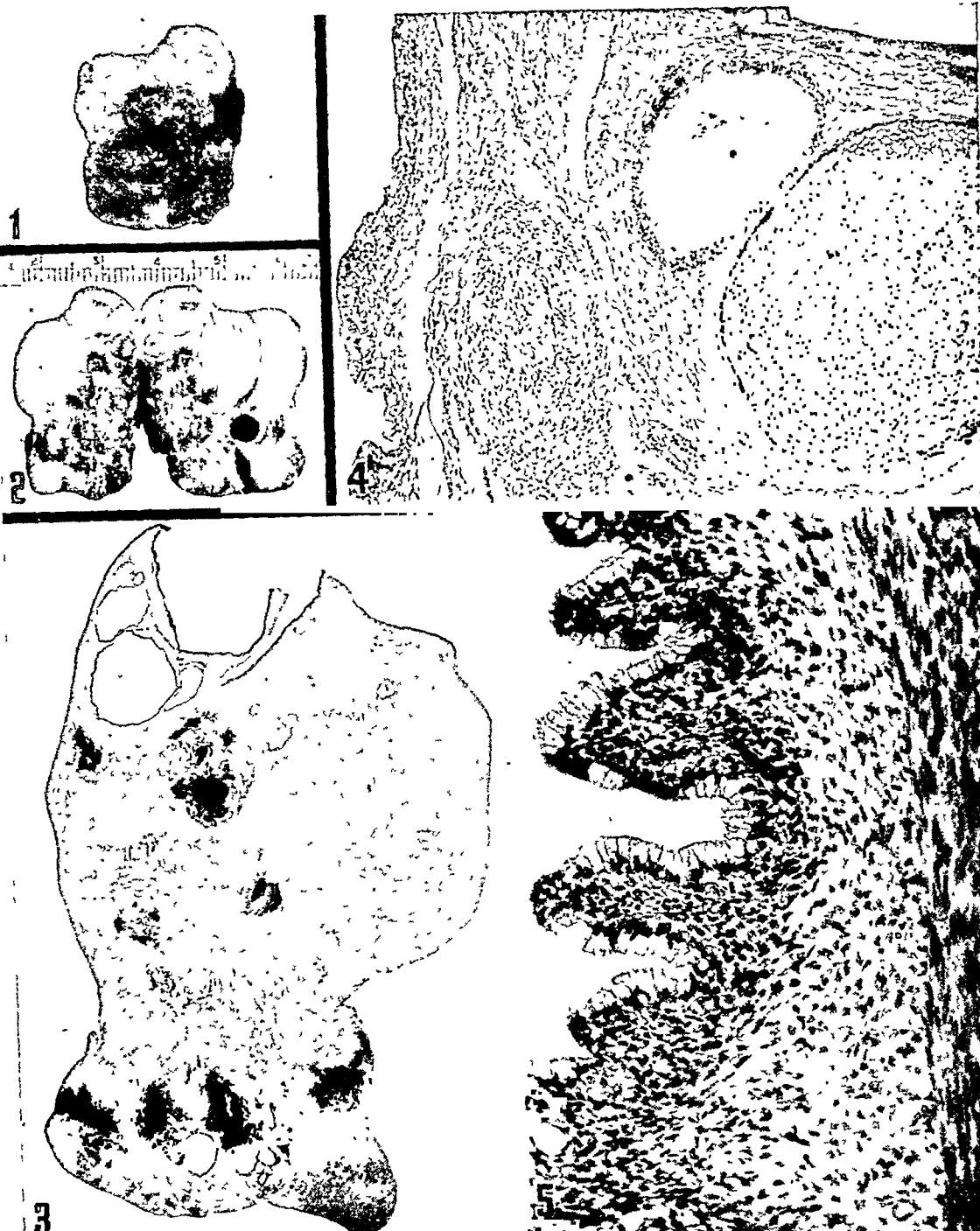


Fig. 1.—Tumor as removed.

Fig. 2.—Tumor opened in sagittal plane.

Fig. 3.—Sagittal section of tumor.

Fig. 4.—Low power magnification of a cystic portion of the tumor showing cysts lined with mucus-secreting epithelium and a mass of cartilage in the connective tissue septum.

Fig. 5.—High power magnification of a cyst wall showing smooth muscle, submucous connective tissue and columnar mucus-secreting epithelium.

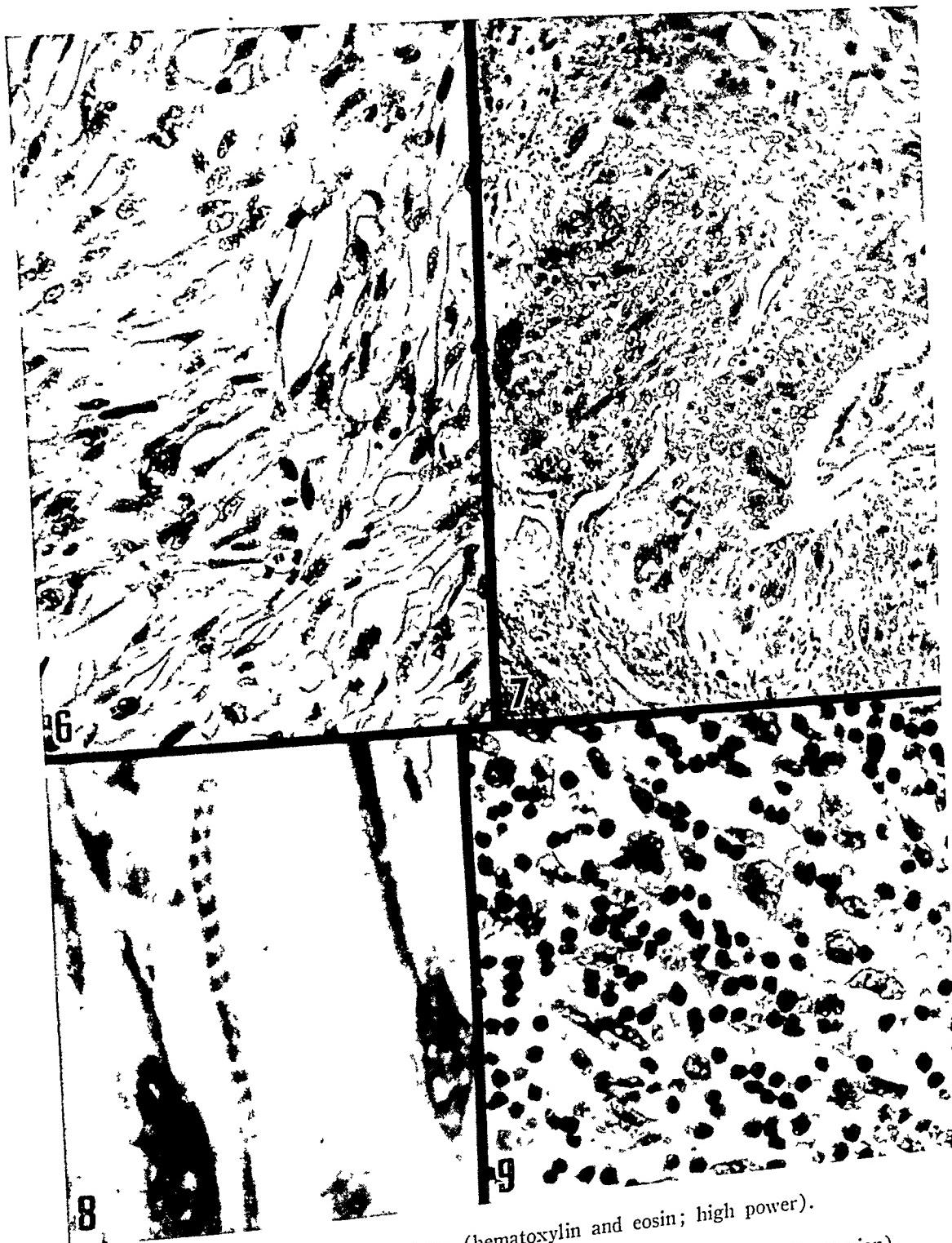


Fig. 6.—Rhabdomyosarcoma (hematoxylin and eosin; high power).

Fig. 7.—Choriocarcinoma (high power).

Fig. 8.—Striated muscle fiber (Heidenhain's iron-hematoxylin; oil immersion).

Fig. 9.—Area resembling classic "pinealoma" (high power).

There was bilateral papilledema of about 4 diopters. The lower part of the left side of the face and the left limbs seemed to be a little weak. The Babinski reflex was positive on the left. The neck was slightly rigid. In all other respects the examination gave negative results.

A general examination showed nothing of special note. The genitalia were of the normal adult male type. The urine and the blood were normal. The Wassermann, Kahn and Kline tests were negative. Roentgenograms of the skull showed some irregular calcification in the region of the pineal gland.

Ventriculography was performed August 1, about 100 cc. of fluid being removed and replaced with air. The roentgenograms showed a large filling defect in the posterior half of the third ventricle, apparently caused by a pineal tumor. The anterior half of the third ventricle and both lateral ventricles were dilated.

August 5 a transventricular approach to the tumor was attempted unsuccessfully after turning down a right parieto-occipital osteoplastic flap. A generous decompression was made at the base of the flap, and the incision was closed. August 17 it was noted that the convergence spasm was considerably less marked and that the papilledema had receded to about 2 diopters in each eye.

August 24 the osteoplastic flap was reelevated and the inner half of the right occipital lobe resected. A tumor about the size of a golf ball was found just beneath the splenium of the corpus callosum. It was yellowish gray, firm in consistency, irregularly nodular and well encapsulated. It was separated carefully from surrounding structures and removed in one piece. The bone flap was replaced and the incision closed.

His general condition was satisfactory the afternoon and evening of the day of operation. The following morning he had an attack of opisthotonus. A spinal puncture was performed, bloody fluid being obtained. Successive attacks of opisthotonus occurred. His general condition became progressively poorer. Death occurred August 27.

*Gross Specimen.*—The tumor was ovoid, 33 by 31 by 30 mm. The outer surface was generally rough and hemorrhagic except for a rounded glistening area at one pole overlying a cystic portion. On cut section three types of tissue were found. One was composed of small cysts; another, of solid white firm tissue, and the larger part, of red, granular, seminecrotic tissue. Some cysts contained clear fluid; others, mucus.

*Microscopic Examination.*—A sagittal plane section showed several epithelium-lined cysts. Some were lined with mucus-secreting columnar epithelium resembling intestinal mucosa; others showed ciliated epithelium resembling respiratory mucosa. The white firm area of the tumor was made up of irregular spindle-shaped cells of an immature type which on further study proved to be rhabdomyosarcoma; cross striations were demonstrated in the fibers of the cytoplasm of the spindle-shaped cells. The hemorrhagic areas of the tumor were necrotic except for a few islands of tissue present which exhibited masses of syncytial cells in conjunction with proliferating Langhan's cells. A very small area was suggestive of the usual "pinealoma." This area merged almost imperceptibly into the area of sarcoma. Spherules of calcium were found in the myosarcoma and in the hemorrhagic tissue of the choriocarcinoma.

*Autopsy.*—The autopsy showed no significant additional features. The tumor had been completely removed. No pineal tissue was found. The autopsy included the abdomen and the thorax. Unfortunately, neither the testicles nor the prostate were examined microscopically. The lungs showed patchy pneumonia and no metastasis.

# Laboratory Methods and Technical Notes

## A PORTABLE EXHIBIT CASE

GEORGE J. RUKSTINAT, M.D., CHICAGO

THE NEED for a portable case in which to exhibit Translite photographs, to supplement the usual demonstration shelf for preserved specimens in the pathologic museum, prompted the designing of the device described here. The general utility has been expanded to include its being used in classrooms and clinics and at conventions of medical societies.



Fig. 1.—The case packed for shipping, showing a rear view.

Basically the case is a sturdy sectional contrivance, constructed of readily available materials. The boxlike base is 42 inches (106.5 cm) high and wide. Its front is common plywood,  $\frac{3}{8}$  inch (about 1 cm) thick, and the sides, the

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From the Department of Pathology, Presbyterian Hospital.

top and the bottom are pine,  $\frac{7}{8}$  inch thick and 12 inches wide (about 2 cm. thick and 30.6 cm. wide). The back is open so that it can receive the top when the case is to be transported. Four metal braces 8 inches by  $1\frac{1}{2}$  inches by  $\frac{1}{8}$  inch (about 20 by 4 by 0.3 cm.) reenforce the corners in back and are screwed to the sides and the top or the bottom (fig. 1) and to the viewing top when packed for shipping. They lend rigidity to the structure, keep the top safely within the base and provide a grip for carrying.

The viewing case is  $38\frac{1}{2}$  inches wide and high and  $10\frac{1}{8}$  inches deep (about 97.5 and 27.5 cm.). Its sides, top and bottom are of pine  $\frac{7}{8}$  inches thick. The front is made of a hinged and locked window sash with twelve lights, 10 inches high and 8 wide (about 25.5 and 20 cm.) (fig. 2). The sides and the top of the



Fig. 2.—Front view of top section, showing arrangement of lights and film spaces.

sash are  $2\frac{1}{2}$  inches wide and the bottom is  $3\frac{1}{2}$  inches wide (about 6 and 9 cm.). The sash is mounted so that the recesses, to receive the panes, are inside. In actual use a Translite film is mounted between two panes of single thickness glass, and the panes are bound together with gummed paper tape. The photograph is then held in place by small strips of molding screwed to the sash (fig. 3). Descriptions of the films are made on 6 inch by 1 inch (14 by 2.5 cm.) cards slipped into a holder made up of two strips of molding under each.

The inside of the case was given two coats of aluminum paint to enhance reflection from the twelve lights which are mounted on the back and centered so

that each will primarily light one photograph in the front. The diffusion from other lights minimizes spot lighting of the film. The lights are common Mazda lamps of 60 watts each, set in porcelain bases, which are connected in parallel by asbestos-insulated wire.

Escape of heat is provided for by twenty-four holes  $\frac{1}{2}$  inch (about 1 cm) in diameter, which were drilled into the top at a 45 degree angle to minimize escape of light and entrance of dust. The bottom is set up 2 inches (5 cm) from the lower ends of the sides in order to provide a safe recess for the switch which controls the lights and also to allow a space for the circulation of air. An opening for air in the bottom is 2 inches by 20 inches (5 cm by 51 cm), placed as a long slit near the middle.



Fig. 3.—A close-up view of the interior of the viewing section, showing molding cleat to retain film.

The back of the viewing box is made of  $\frac{3}{8}$  inch plywood and contains a single 2 inch hole through which the electric plug and wire connection to a current source can be retracted into the case during shipment. This back also protects the lighting connections and glassed sash during shipment (fig 1). The outside of both base and viewing box were given two coats of flat black paint. Together they weigh 93 pounds (42 Kg.), the top alone, 54 pounds (24.5 Kg.). As used at present, the top is simply set on the base and plugged into the electric current. Mounted on casters and permanently assembled, the case can serve many departments, and the base can be used to store a variety of exhibits.

## IMPROVED METHODS FOR DEMONSTRATING AMYLOID IN PARAFFIN SECTIONS

BENJAMIN HIGHMAN, M.D., BETHESDA, MD.

THE PRESENCE of amyloid in paraffin sections may be demonstrated by certain metachromatic aniline dyes. For this purpose, the deparaffinized hydrated sections are commonly stained with aqueous solutions of methyl violet or crystal violet, often followed by differentiation in dilute acids.<sup>1</sup> The violet red metachromatic color imparted to the amyloid by these dyes is changed to blue by such dehydrating agents as acetone, dioxane and the various alcohols which are commonly used before clearing in xylene and mounting in balsam or clarite. Hence, the hydrated stained sections are usually mounted in water, glycerin, syrup or saturated aqueous solution of fructose or potassium acetate.

In skilled hands, such staining methods are generally satisfactory. However, water, glycerin and nonsetting aqueous solutions are unsatisfactory as permanent mounting mediums. If glycerin or Apáthy's syrup is used, there is frequently noted within a short period marked fading or obscuring of detail due to diffusion or bleeding of the stain into these mounting mediums. Furthermore, nuclei are often poorly stained, and considerable care is required to prevent understaining or excessive decolorizing by the acid and to prevent overstaining or insufficient differentiation. Overstaining changes the color of the amyloid to purple or blue and lessens the contrast.

These faults can be corrected to a large extent by using the following technic:

Deparaffinize sections in xylene and pass through graded alcohols to water in the usual manner.

Stain five minutes with hematoxylin, preferably by Weigert's iron-hematoxylin-acid method, then wash in water.

Stain one to five minutes in a 0.5 per cent solution of crystal violet or methyl violet in 2.5 per cent acetic acid.

Wash in water and mount in the modified Apáthy's syrup described in a later paragraph.

This technic has been used successfully on a great variety of tissues fixed with formaldehyde. Amyloid, cartilage and certain types of mucoid material were stained purplish to violet red against a bluish background. Nuclei were stained sharply by the hematoxylin.

From the Pathology Laboratory, National Institute of Health, United States Public Health Service.

1. (a) Lillie, R. D., cited by Conn, H. J., and Darrow, M. A.: Procedures Used by the Biological Stain Commission, Geneva, N. Y., Biotech Publications, 1943. (b) Mallory, F. B.: Pathological Technique, Philadelphia, W. B. Saunders Company, 1938. (c) Conn, H. J., and others: Biological Stains, ed. 4, Geneva, N. Y., Biotech Publications, 1940. (d) Romeis, B.: Taschenbuch der mikroskopischen Technik, ed. 13, Munich, R. Oldenbourg, 1932.

Prestaining with hematoxylin improved nuclear staining markedly. The acetic acid added to the staining solution greatly retarded overstaining without fading the hematoxylin appreciably and thus permitted a wide range of staining time. Good results were obtained by staining for periods of from as little as thirty seconds in a 0.1 per cent solution of the dye to as long as thirty minutes in a 0.5 per cent solution of the dye in 2.5 per cent acetic acid. Certain other acids, such as dilute to concentrated hydrochloric acid and formic acid, exerted a similar retarding influence on overstaining by the aniline dyes, but they caused fading of the hematoxylin after a few minutes. When 2.5 per cent oxalic acid was used in place of acetic acid, fading of the hematoxylin was rapid, but nuclear staining by the crystal violet was fair, so that the hematoxylin could be omitted if sharp nuclear staining was not required.

There was no appreciable diffusion or bleeding of the dye when saturated aqueous solutions of fructose or potassium acetate were used as mounting medium. Diffusion was quite marked in glycerin and less so in Apáthy's syrup prepared as described by Mallory<sup>1b</sup> or as modified by Lillie and Ashburn.<sup>2</sup> As modified by the latter authors this syrup consists of 50 Gm. each of acacia and cane sugar dissolved in 100 cc. of water with 1.5 cc. of 1 per cent merthiolate added as a preservative. Bleeding into this medium can be prevented or minimized by adding 50 Gm. of potassium acetate. A smaller amount of potassium acetate was less effective, and a larger amount greatly delayed the setting of this mounting medium. Certain other crystalloids such as sodium and calcium chloride, sodium acetate, ammonium nitrate or potassium citrate, exerted a similar action against diffusion of the stain when they were dissolved in the mounting medium. Recently we have been adding 10 Gm. of sodium chloride in place of the potassium acetate. Results are good, and the chloride interferes less with the hardening of the syrup than does 50 Gm. of potassium acetate. Attempts to preserve the metachromasia of hydrated stained sections in balsam or clarite have proved unavailing.

Another widely used method for demonstrating amyloid in paraffin sections is Bennhold's congo red stain<sup>3</sup>: The deparaffinized and hydrated sections are stained ten to twenty minutes or longer in a 1 per cent aqueous solution of congo red, dipped fifteen seconds in a saturated aqueous solution of lithium carbonate, decolorized in 80 per cent alcohol until clouds of stain no longer come from the sections, washed in water fifteen minutes, counterstained with alum-hematoxylin solution, washed in water, dehydrated in 95 per cent and absolute alcohol, cleared in xylene and mounted in balsam. The results are often excellent. However, the time consumed is rather long, and the amyloid may be largely decolorized if differentiated in alcohol more than a few minutes.

I have found the following modification quicker and better:

Stain deparaffinized sections until deep red with 0.5 per cent congo red or congo corinth G (color index no. 375) in 50 per cent alcohol. Usually one to five minutes is adequate.

2. Lillie, R. D., and Ashburn, L. L.: Arch. Path. 36:432, 1943.

3. Bennhold, H.: München. med. Wchnschr. 2:1537, 1922.

Wash in water and differentiate one to three minutes in 80 per cent ethanol containing about 0.2 per cent potassium hydroxide. Rinse in water and counterstain with hematoxylin.

Wash in water, dehydrate, clear and mount by successive use of acetone, acetone-xylene, xylene and clarite.

The congo red mixture was prepared by adding an equal volume of alcohol to a 1 per cent aqueous solution of congo red. As the concentration of the alcohol in the staining mixture was increased up to 85 per cent, the time required for staining by a given concentration of dye was reduced. Perhaps this acceleration of staining is due to a relatively decreased affinity of the dye for the solvent, since the solubility of the dye in the solvent is diminished as the percentage of alcohol is increased. No marked difference in acceleration was noted between 50 per cent acetone, dioxane, methanol, ethanol, propanol, isopropanol and tertiary butanol. Alkalies, such as potassium hydroxide or disodium phosphate, were less efficient than alcohols as accelerators and when present in effective concentrations tended to loosen the sections from the slides.

In comparison with lithium carbonate followed by 80 per cent ethanol as used in the Bennhold method, 80 per cent ethanol with added potassium hydroxide differentiated the sections stained with congo red about as rapidly and decolorized the amyloid more slowly. Thus, in many series of paraffin sections the amyloid was decolorized by the Bennhold method in less than thirty minutes but retained considerable congo red even after an overnight exposure in ethanol containing potassium hydroxide. In a few series, however, the amyloid lost its color by either method within a few minutes, though somewhat more slowly in the 80 per cent alcohol containing potassium hydroxide. Decolorization was less rapid when the alcohol was more dilute.

Other substances, such as corpora amylacea of the prostate or brain, elastic fibers and some cornifying epithelial cells often retained the congo red stain for a variable period after differentiating in alcohol. No marked difference was noted between the two methods in the length of time the congo red was retained by such substances. Congo corinth G (also called Erie garnet B), which is closely related chemically to congo red, could be substituted for the latter but had no particular advantages. The sample used was lot NGe-2 of National Aniline and was labeled Erie garnet B.

Hematoxylin may be used either before or after the congo red stain. Either alum-hematoxylin or iron-hematoxylin may be used, depending on preference for nuclear color.

#### SUMMARY

In one of the two improved methods of staining amyloid in paraffin sections of tissues fixed in solution of formaldehyde the deparaffinized hydrated sections are stained five minutes with iron-hematoxylin to bring out nuclear detail, washed in water, stained one to five minutes in a 0.5 per cent solution of crystal violet or methyl violet in 2.5 per cent aqueous solution of acetic acid, washed in water, and mounted in Lillie's Apáthy's syrup, modified by dissolving 50 Gm. of postassium acetate or 10 Gm. of sodium chloride in 100 cc. of the syrup.

In the second method the deparaffinized sections are stained one to five minutes in 0.5 per cent congo red or congo corinth G in 50 per cent alcohol, differentiated one to three minutes in 80 per cent alcohol containing 0.2 per cent potassium hydroxide, counterstained with alum-hematoxylin or iron-hematoxylin, washed in water, and dehydrated, cleared and mounted by successive use of acetone, acetone-xylene, xylene and clarite.

## Notes and News

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**Appointments.**—Lawrence W. Smith, formerly professor of pathology in Temple University School of Medicine, Philadelphia, is now medical director of the Commercial Solvents Corporation, New York.

Colonel Raymond O. Dart, deputy chief surgeon for the Army Forces in the Western Pacific, has been appointed assistant director of administration at the Army Institute of Pathology. Colonel Dart has been awarded the Legion of Merit and the Bronze Star for his services since going to the Pacific in 1942 as commanding officer of the 105th General Hospital.

Alfred Golden, Washington, D. C., has been appointed assistant professor of pathology at the University of Tennessee College of Medicine, Memphis, Tenn., and director of laboratories at the Baptist Memorial Hospital, Memphis.

Lieutenant Colonel P. J. Melnick, Medical Corps, Army of the United States, on release from active duty was appointed associate professor of pathology at the University of Southern California School of Medicine.

L. S. King has been appointed pathologist and director of laboratories at the Illinois Masonic Hospital, Chicago.

L. C. Kress, formerly director of the cancer division of the New York State Department of Health, is now director of the Roswell Park Memorial Institute, until recently known as the State Institute for the Study of Malignant Diseases, Buffalo.

Valy Menkin, assistant professor of pathology at Duke University School of Medicine, has been appointed associate professor of experimental pathology in the newly created department of surgical research at Temple University School of Medicine in Philadelphia.

Peter Gruenwald has been appointed assistant in pathology in the Long Island College of Medicine, Brooklyn.

**Awards.**—Ernest W. Goodpasture, professor of pathology and dean of Vanderbilt University School of Medicine, is the recipient of the Passano Foundation Award for 1946, established in 1944 by the Williams & Wilkins Company, medical publishers, Baltimore. Dr. Goodpasture was given the award for his "original development of the method for propagation of viruses in pure culture by inoculation of chick embryos and for his outstanding contributions to advancement of knowledge of the cell parasite relationship in bacterial and virus infection."

Colonel E. R. Long, chief consultant in tuberculosis, Surgeon General's Office, has been awarded the Legion of Merit for his development of standards for the detection of tuberculosis, including his service in creating "priceless permanent files of chest x-ray films of army personnel."

**Society News.**—The new officers of the American Association for Cancer Research are William U. Gardner, New Haven, Conn., president; J. J. Bittner, Minneapolis, vice president; C. W. Hooker, New Haven, Conn., acting secretary-treasurer.

P. R. Cannon, University of Chicago, R. F. Loeb, Columbia University and E. R. Long, University of Pennsylvania, have been elected members of the National Academy of Sciences.

**Public Health Laboratory Fellowships.**—Five fellowships have been established by the New York State Department of Health for the training of physicians in the public health laboratory field. The appointments are for a period of one year and are open to physicians with postgraduate laboratory training who wish additional experience in pathology, bacteriology or biochemistry to qualify for positions in the public health laboratories in New York. Candidates must be United States citizens who have graduated from a medical school approved by the American Medical Association and who are licensed or eligible to take the examination for license to practice medicine in New York state. Inquiries should be directed to the Division of Laboratories and Research, State Department of Health, Albany 1, New York.

**Children's Cancer Center.**—A service to assist in the diagnosis and treatment of tumors in early life has been organized in the Medical Center for Children in Boston. The object is to give free diagnostic assistance and advice to any physician who will send the necessary data, sections and roentgenograms. The project, which includes research, is supported by a grant from the National Cancer Institute. The consultants are S. B. Wolbach, C. F. Branch and S. Farber, pathologists, and E. B. D. Neuhauser, roentgenologist, and members of the staff of the Children's Hospital.

**Death.**—Milton J. Rosenau, outstanding for his work in public health and preventive medicine, died April 9, 1946, 77 years old. He was surgeon in the United States Public Health and Marine Hospital Service from 1890 to 1909. From 1909 to 1935 he was professor of preventive medicine and hygiene in Harvard Medical School and from 1922 to 1935 also professor of epidemiology in the Harvard School of Public Health. In 1936 he went to the University of North Carolina, where he directed the new school of public health and was professor of epidemiology. Of Rosenau's researches, the pioneer work by him and Anderson on human anaphylaxis deserves special mention.

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## Books Received

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**SYNOPSIS OF PATHOLOGY.** By W. A. D. Anderson, M.A., M.D., F.A.C.P., professor of pathology and bacteriology, Marquette University School of Medicine; pathologist, St. Joseph's Hospital, Milwaukee. Second edition. Pp. 722, with 327 illustrations and 15 color plates. Price, about \$6. St. Louis: C. V. Mosby Company, 1946.

The first edition was published in 1942. Additions and betterments have been made in practically every chapter. There are some acceptable new illustrations. Colored plates 3, 6, 8 and 15 are decidedly below par. Why should not the larynx receive consideration as well as other parts of the respiratory system? In 1943, 1,490 deaths from cancer of the larynx occurred in this country. In the paragraph on epidemic hepatitis (p. 384) nothing is said about the modes of its causation, and in the statement about acute yellow atrophy of the liver (p. 382) no mention is made of its relation to epidemic hepatitis. But in view of the extent of the book it seems remarkably free from shortcomings. It gives a helpful epitomé of pathology as presented in current textbooks. The morphologic aspects, gross and microscopic, are well described and illustrated.

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## LOCAL TISSUE REACTIVITY (SHWARTZMAN PHENOMENON) IN THE HEART AND THE FEMORAL ARTERY OF THE RABBIT

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IN THE production of the phenomenon of local tissue reactivity (Shwartzman<sup>1</sup>) a new category of toxic antigens has been demonstrated in bacterial "agar washing" filtrates obtained from a variety of pathogenic micro-organisms. From the standpoint of physiochemical properties these antigens are similar to the usual bacterial exotoxins; yet the tissue response which they elicit in the experimental animal differs from that observed after the introduction of bacterial exotoxins prepared in the usual way. The usual toxins inflict damage in direct proportion to their strength; this is true also for the bacterial "agar washing" filtrates, but in addition it has been shown that when a local injection of a subtoxic dose of filtrate is followed twenty-four hours later by an intravenous injection of the same filtrate diluted to such a point as to be unable per se to produce any remarkable tissue reaction, a striking necrotic-hemorrhagic inflammatory change is produced at the site of the local injection.

This course of events, first described in the skin,<sup>2</sup> has been uniformly produced in many parts of the body: lungs,<sup>3</sup> stomach,<sup>4</sup> knee joints,<sup>5</sup> appendix,<sup>6</sup> adrenal glands,<sup>7</sup> conjunctivas,<sup>8</sup> pancreas,<sup>9</sup> kidneys<sup>10</sup> and

From the Mount Sinai Hospital Laboratories (Dazian Fellow), New York.

1. Shwartzman, G.: The Phenomenon of Local Tissue Reactivity, New York, Paul B. Hoeber, Inc., 1937.
2. Shwartzman, G.: J. Exper. Med. **48**:247, 1928.
3. Shwartzman, G.: J. Exper. Med. **51**:571, 1930.
4. Karsner, H. T.; Ecker, E. E., and Jackson, E. L.: Proc. Soc. Exper. Biol. & Med. **29**:319, 1931.
5. Moritz, A. R., and Morley, J. D.: Proc. Soc. Exper. Biol. & Med. **29**:321, 1931.
6. Latteri, S.: Riv. di pat. sper. **13**:389, 1934.
7. Gronchi, V.: Boll. Soc. ital. di biol. sper. **9**:1032, 1934.
8. Cassuto, N.: Sperimentale, Arch. di biol. **87**:191, 1933.
9. Reitano, R., and Loi, L.: Boll. Soc. ital. di biol. sper. **9**:196, 1934.
10. Shwartzman, G.; Bernheim, A. I., and Gerber, I. E.: J. Mt. Sinai Hosp. **4**:1020, 1938.

neoplastic tissue.<sup>11</sup> In all these experiments, regardless of the site and of the method of preparation, the development of the phenomenon was consistently characterized by a severe vascular response with hemorrhages, extreme venous dilatation and engorgement, and thrombosis of venules and capillaries. Since an explanation of this peculiar manifestation could not be found on purely morphologic ground, it was thought that a condition of endothelial hypersensitivity initiated by the local preparatory injection and activated by the reacting factors reaching the vessels by way of the blood stream accounted both for the vascular damage and for the hemorrhagic tissular change.

The present investigation is mainly concerned with the phenomenon as observed histologically in the heart and the femoral artery. Since the procedures involved in these experiments established conditions that might be compared in certain aspects with those studied by others in artificially produced allergic states and with carditis and angiitis induced by bacterial toxins, a comparative analysis will be made and integrated with some further observations in which the effect on the heart and a peripheral artery of a non-phenomenon-producing substance was studied.

#### MATERIAL AND METHODS

The experiments were divided into the following two main groups: (a) the production of the phenomenon in the heart and a femoral artery by the use of a bacterial filtrate, and (b) duplicate experiments in which rheumatic blood was used. In the first group of experiments these procedures were followed:

1. A single intracardiac injection of bacterial filtrate (18 rabbits).
2. A single intravenous injection of the filtrate (8 rabbits).
3. An intracardiac injection followed by an intravenous injection (16 rabbits)—the cardiac Shwartzman phenomenon.
4. A local periarterial and an intra-arterial injection (9 rabbits).
5. A local periarterial injection followed by an intravenous injection (3 rabbits)—the periarterial Shwartzman phenomenon.
6. A local intra-arterial injection followed by an intravenous injection (3 rabbits)—the intra-arterial Shwartzman phenomenon.
7. A combined arterial and cardiac phenomenon (4 rabbits).

Stock rabbits of an average weight of .2 Kg. were used as experimental animals. "Agar washing" filtrates of a twenty-four hour old culture of meningococcus (type B, group 3) prepared according to the method described by Shwartzman were employed both for the local preparatory injection and for the provocative intravenous injection.

For the intracardiac preparation the filtrate was injected directly into the heart through a  $\frac{5}{8}$  inch (1.5 cm.), 25 gage hypodermic needle inserted in a depilated area of intact thoracic wall. The effect of the trauma by itself was determined by a control procedure in 4 animals which were given local and intravenous injections, singly and combined, of saline solution. No evidence of damage was encountered in any of these.

For the arterial preparation the femoral artery was exposed under aseptic technic and the filtrate injected by hypodermic needle either into the periadventitial tissue or directly into the lumen of a double ligated tract of the vessel.

11. Gratia, A., and Linz, R.: Compt. rend. Soc. de biol. 108:427, 1931.

The traumatic effect of the surgical maneuver necessary to prepare the femoral artery and to close the wound was determined in each animal individually by exposing and closing the same vessel of the other extremity and later examining the site both grossly and microscopically.

In all animals the provocative intravenous injection of the filtrate was given in the marginal vein of an ear twenty-four hours after the preparatory injection.

In order to compare the effects of a phenomenon-producing substance (bacterial filtrate) with those elicited by a non-phenomenon-producing substance, the same experimental procedures were repeated in another series of animals with blood from a patient who had rheumatic fever. As in the experimental elicitation of the phenomenon, the necessary control experiments were carried out.

1. A single intracardiac injection of rheumatic blood (2 rabbits).
2. A single injection and double (one week apart) intravenous injections of rheumatic blood (6 rabbits).
3. An intracardiac injection followed by an intravenous injection (6 rabbits).
4. A local intra-arterial injection (3 rabbits).
5. A local intra-arterial injection followed by intravenous injection (3 rabbits).

The intracardiac injection was performed according to the procedures already described, 0.5 cc. of blood being used; 2 cc. was used for the intravenous injection, and for the local arterial preparation several drops of blood was injected into the lumen of a ligated section of a femoral artery. The animals which had received the single intravenous injection, the double intravenous injections and the single intracardiac injection were killed after twenty-four hours. The animals which had received the intracardiac injection followed by an intravenous injection twenty-four hours later were killed, 2 at twenty-four hours, 2 at the third day and 2 at the seventh day. The animals given the single local intra-arterial injection and the local intra-arterial injection followed by an intravenous injection twenty-four hours later were killed after one day.

The patient from whom the blood was obtained had had a recent and severe attack of rheumatic fever with cardiac involvement and generalized swelling of the large joints. When the samples of blood were taken, the disease process appeared to be in remission, and the temperature was normal. Repeated blood cultures were constantly negative. Control experiments were carried on in a few rabbits, blood from normal persons being used. The impression obtained was that the microscopic findings were somewhat different from those noted in the rabbits inoculated with the blood of the rheumatic patient; however, since this point will be investigated further, no conclusions are made at present.

#### THE CARDIAC AND ARTERIAL SHWARTZMAN PHENOMENON

##### *1. Effect of a Single Intracardiac Injection of Bacterial Filtrate.*

In order to obtain a dilution of filtrate of such minimal strength as to be unable to elicit any remarkable tissue response on being injected into the myocardium once, increasing dilutions of filtrate were given in a fixed amount (0.3 cc.) to a series of 18 rabbits. Four animals received undiluted filtrate and were killed at the twenty-fourth hour. Fibrinous pericarditis and exudative myocarditis, with polymorphonuclear leukocytes infiltrating the involved tissues to a striking degree, were consistent findings in all 4, and in 1 a firmly adherent mural thrombus was found implanted on the endocardium lining the left ventricle. Similar changes were shown by 3 other animals which had received dialyzed and frozen filtrate diluted 1:3. The same filtrate at the dilution of 1:20 was found to be productive

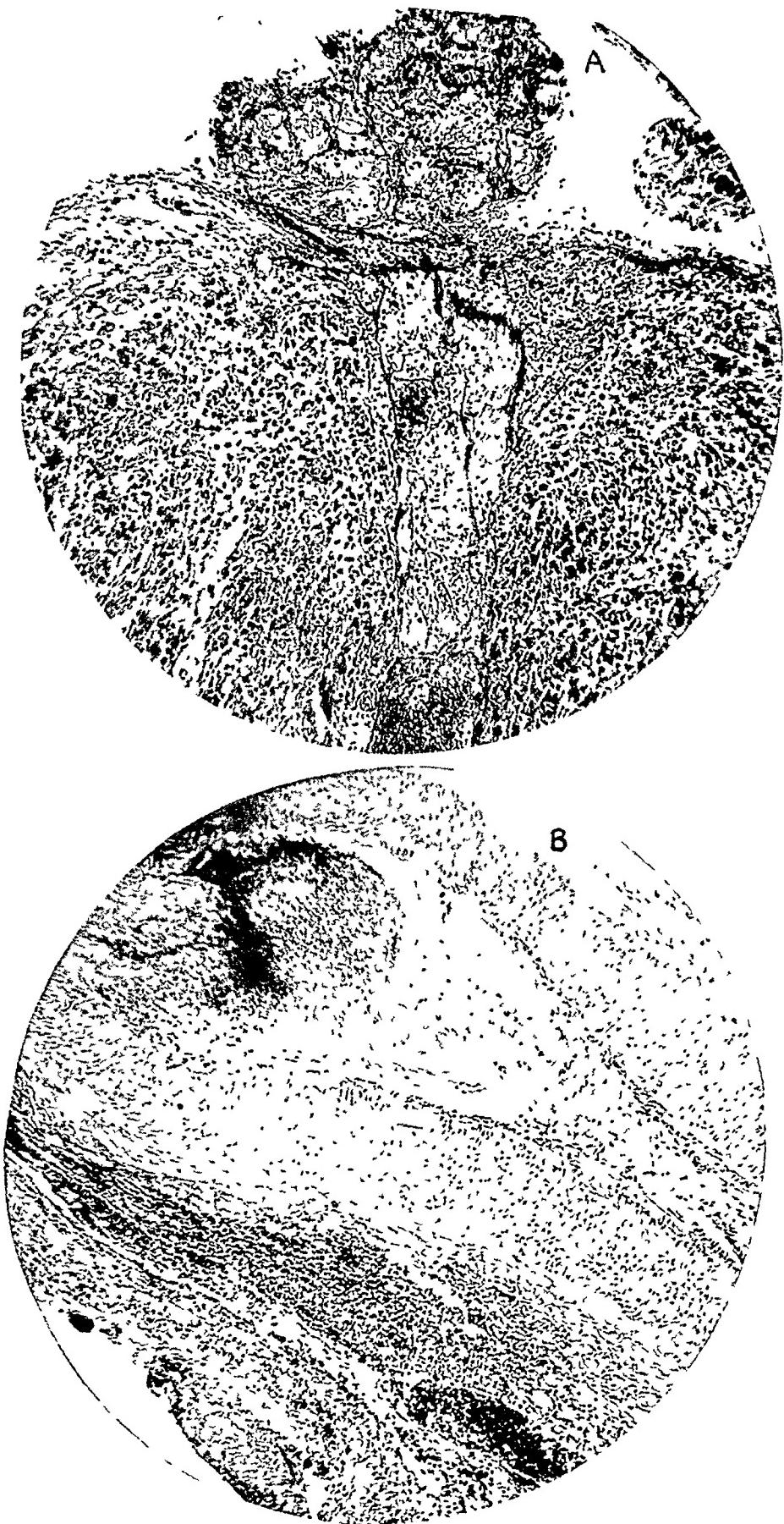


Figure 1  
(See legend on opposite page)

of a much milder reaction in 2 other animals, and an inconstant negligible tissue response followed the injection of dialyzed filtrate diluted 1:30 in 9 rabbits. The 1:30 dilution of the filtrate was therefore considered satisfactory as the preparatory dose and was used as such in the production of the phenomenon.

### *2. Effect of a Single Intravenous Injection of the Filtrate.*

The effect of a single intravenous injection of bacterial "agar washing" filtrate on the heart as well as on the other organs has been analyzed by Gerber.<sup>12</sup> A few trials enabled us to confirm his observations (figs. 2B and 3A). Four rabbits were given an intravenous injection of 2 cc. of filtrate diluted 1:10. In these animals the only evidence of cardiac damage was a waxy appearance in groups of myofibrils and a moderate interstitial inflammatory reaction, mostly on the part of polymorphonuclear leukocytes. In 4 other rabbits given an injection of the same filtrate dialyzed and diluted 1:20, no evidence of myocardial damage was found, and this dilution was therefore thought satisfactory for the provocative injection.

### *3. Effect of the Intracardiac Injection Followed by an Intravenous Injection.*

Five rabbits received plain bacterial "agar washing" filtrate; 11 received dialyzed and frozen bacterial "agar washing" filtrate. Regardless of the material used, all animals showed an identical pattern of lesions.

**Gross Findings.**—In 10 animals killed twenty-four hours after the provocative intravenous injection, layers of a flaky fibrinous exudate were found on both the epicardial and the pericardial membrane. There were concomitant hemorrhagic changes, either limited to the site of the local preparatory injection or massive in distribution and involving the entire pericardium in a mottled fashion. The epicardial vessels were visibly engorged, and petechial hemorrhages outlined their course.

The underlying myocardium presented a markedly variegated appearance owing to alternating yellowish gray, dry, friable areas grossly suggesting necrobiotic change and reddish brown hemorrhagic areas involving the entire thickness of the myocardium to the parietal endocardium. In 3 of these 10 animals, large thrombotic masses firmly adherent to the parietal endocardium bulged into the left ventricular cavity. The thrombi were reddish brown, showed irregularly contracted surfaces and exhibited on the cut section a layered appearance. One of these animals had concomitant involvement of the tricuspid valve, the anterior leaflet of which was hemorrhagic and edematous in appearance.

The 6 other rabbits, dying from two to five hours after the intravenous injection of the filtrate, displayed similar pericardial and myocardial changes, and in 2 of these mural thrombi were implanted on the wall of the left ventricle.

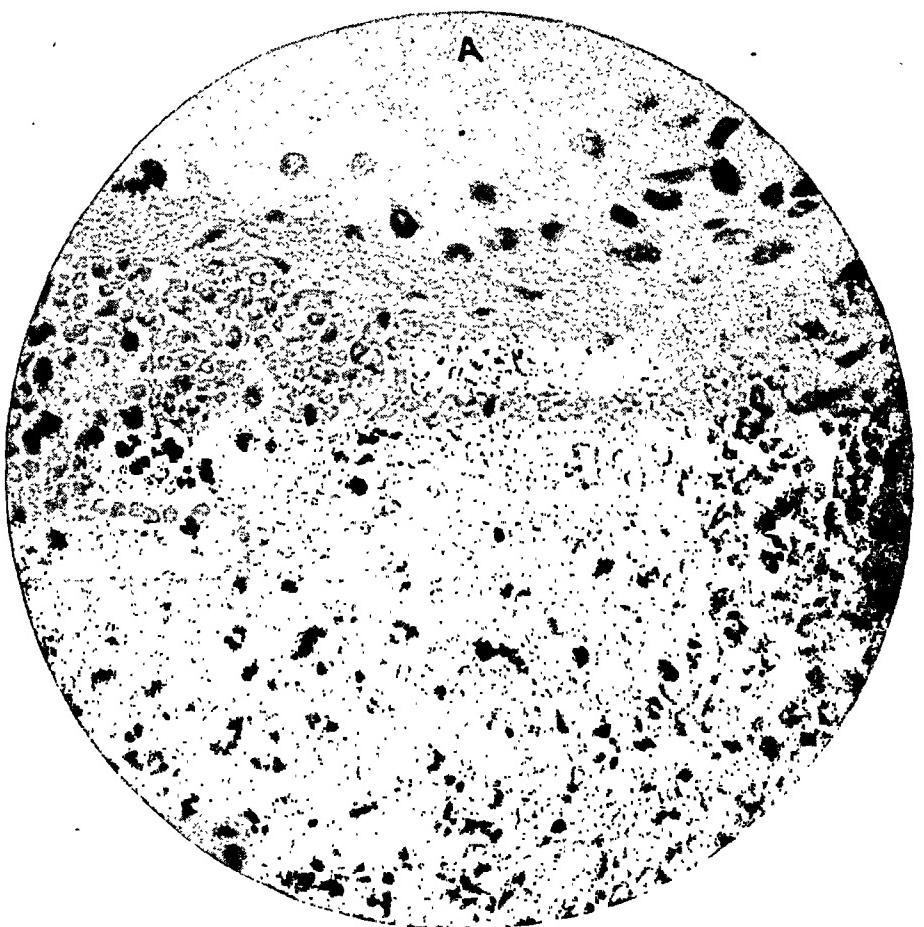
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12. Gerber, I.-E.: Arch. Path. 21:331 and 776, 1936.

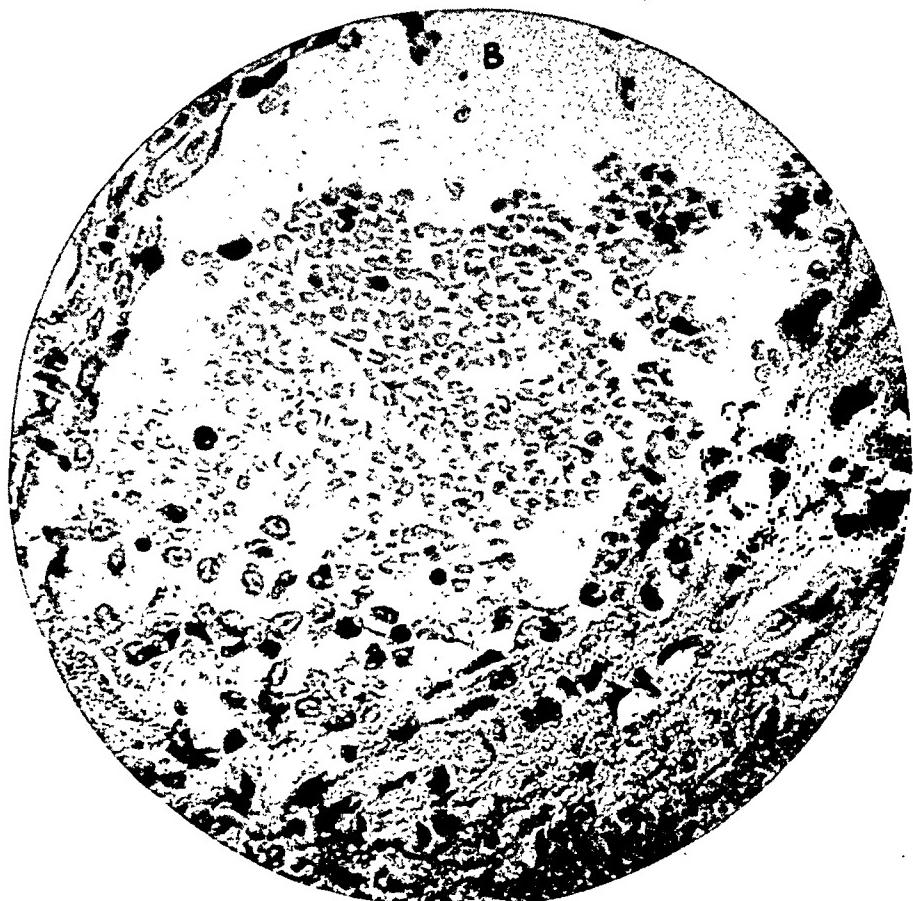
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Fig. 1.—*A*, parietal endocardial thrombosis, venous dilatation and engorgement, and necrotic-hemorrhagic myocardial change, with polymorphonuclear leukocytes infiltrating the tissues, occurring at the site of an intracardial injection of diluted bacterial infiltrate twenty-four hours after an intravenous injection of the same filtrate—the cardiac Shwartzman phenomenon. (Photomicrograph, ocular 10, objective 8, Zeiss.)

*B*, mural thrombosis, severe necrotic-hemorrhagic arteritis and periarteritis in a doubly ligated tract of femoral artery occurring at the site of an intraluminal injection of diluted bacterial filtrate twenty-four hours after an intravenous injection of the same filtrate—the intra-arterial Shwartzman phenomenon. (Photomicrograph, ocular 10, objective 8, Zeiss.)



A



B

Figure 2  
*(See legend on opposite page)*

*Microscopic Changes.*—Widespread congestion of capillaries and venules, with recently extravasated red blood cells extensively infiltrating the adjacent tissues, was striking both in the myocardium and in the pericardium. Numerous small and middle-sized veins were thrombosed. The thrombi varied from parietal clumps of hematic cells embedded in the meshes of a fibrinous material to larger amorphous eosinophilic masses obliterating the lumen almost completely. The vessel walls displayed severe necrobiotic changes and abundant leukocytic infiltration, most severely affecting the media and the adventitia. Intimal damage was apparent in the thrombosed tracts of blood vessels, but aside from these areas, in the majority of the vessels encountered the intima appeared fairly well preserved, with endothelial lining and elastic structures being clearly made out. In the hemorrhagic areas, the myofibrils displayed striations and nuclear patterns that were hardly recognizable, and often they appeared fused into amorphous blocks of structureless eosinophilic material. Identical necrobiotic myocardial changes were noticeable at times in other areas apparently free from hemorrhagic infiltration. Proliferating histiocytes, macrophages containing fragments of red blood cells and overwhelming numbers of infiltrating polymorphonuclear leukocytes were constant additional observations. Few leukocytes appeared well preserved; the majority showed karyorrhexis and karyolysis resulting in a scattering of cellular debris. The endocardial parietal thrombi consisted mostly of masses of eosinophilic material which merged indistinctly into the underlying necrotic structureless myocardium (fig. 1A). In the anterior leaflet of the tricuspid valve, which had grossly appeared hemorrhagic and edematous, the histologic preparations revealed focal areas of necrosis in the ground substance with loss of fibrillar structure and scattering of cellular debris. Widespread extravasations of red blood cells and diffuse infiltration by polymorphonuclear leukocytes almost completely obliterated the usual valvular structure (fig. 2A).

#### 4. Effect of a Local Periarterial and an Intra-Arterial Injection.

All of the control experiments described in the demonstration of the Schwartzman phenomenon in the heart were repeated in the study of the phenomenon in a peripheral artery. After repeated trials, a dilution of the filtrate was obtained which on local injection, either intravascular or perivascular, and on intravenous injection evoked a negligible tissue response in the tract of artery subjected to the double ligation. A dilution of 1:30 was found to be satisfactory for local injection and a dilution of 1:20 for intravenous administration.

#### 5. Effect of a Local Periarterial Injection Followed by an Intravenous Injection of Bacterial Filtrate.

The pattern of lesions in all 3 animals used was quite comparable to that observed in the cardiac phenomenon and made it unnecessary to study the morphologic aspects of the arterial phenomenon in a larger number of animals.

At the prepared tract of femoral artery the periadventitial fibrous connective tissue appeared edematous, with a mottling of reddish brown discoloration and

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Fig. 2.—*A*, acute hemorrhagic valvulitis (tricuspid leaflet) occurring twenty-four hours after an intravenous injection of diluted bacterial filtrate in an animal subjected to intracardiac preparation. (Photomicrograph, ocular 5, oil immersion, Zeiss.)

*B*, hyperplasia of the endothelial lining and swelling of the ground substance of a mitral leaflet of an animal killed twenty-four hours after a single intravenous injection of undiluted bacterial filtrate. (Photomicrograph, ocular 10, objective 40, Zeiss.)

pinpoint hemorrhages which involved the vessel wall to a lesser extent. The lumen was patent, and no evidence of thrombosis could be seen.

On microscopic examination the outstanding abnormality was a mural hemorrhagic-necrobiotic change affecting the periadventitial fibrous connective tissue, the adventitia and the external aspect of the medial coat. Concomitantly there was a diffuse infiltration of polymorphonuclear leukocytes, and several vasa vasorum showed fresh thrombotic masses in their lumens. Similar changes were observed in the walls of adjacent veins. Both in the arteries and in the veins the intimal damage was negligible, the evidence consisting mostly of swelling of the collagenous fibers and some increase of subendothelial histiocytes.

#### *6. Effect of a Local Intra-Arterial Injection Followed by an Intravenous Injection of the Filtrate.*

Three animals were subjected to the procedure. As in the 3 animals of the previous group, a hemorrhagic change affecting both perivascular fibrous connective tissue and vessel wall was observed in all, together with thrombotic occlusion of the lumen of the prepared tract of artery.

The microscopic sections showed the intraluminal thrombi to consist of masses of amorphous eosinophilic material in which platelets, white blood cells and erythrocytes were enmeshed. Wherever the thrombi were implanted, the underlying intima had lost all structural details. The internal elastic membrane was intact, however. The media and the adventitia displayed marked leukocytic infiltration around foci of tissue necrosis (fig. 1B). The adventitia was widely infiltrated by recently extravasated red blood cells, which also invaded the surrounding periadventitial tissue. Aside from the areas of luminal thrombosis, the endothelial cells lining the intima seemed to be well preserved, and the only evidence of damage consisted of moderate endothelial swelling and interstitial edema. Extension of the inflammatory process from the artery to the vein was observed in 1 instance. The wall of the vein was infiltrated by polymorphonuclear leukocytes, and the lumen was completely occluded by a recent thrombotic mass.

#### *7. The Combined Arterial and Cardiac Shwartzman Phenomenon.*

By the double injection technic, in 3 of 4 trials the phenomenon was simultaneously elicited in the intraluminally prepared tract of femoral artery and in the prepared heart. In addition to the striking hemorrhagic-necrobiotic changes in both areas, thrombosis occurred at the arterial site in 2 instances. In a third animal the necrobiotic-hemorrhagic inflammatory reaction was seen to extend from the artery to the adjacent vein, both showing thrombotic occlusion of the lumen. In the fourth animal, the heart displayed the usual pattern of the phenomenon, while the change in the artery was limited to minimal periadventitial lymphocytic infiltration, with occasional granulocytes.

### EFFECT OF RHEUMATIC BLOOD ON THE HEART AND FEMORAL ARTERY

*Gross Findings.*—In none of the animals subjected to this experiment were grossly detectable changes noticed either in the heart or in the prepared stretch of femoral artery.

*Microscopic Changes.*—Microscopic study of the hearts of the animals given a single or two intravenous injections of the rheumatic blood one week apart (group 2) showed generalized proliferation of histiocytes and lymphocytes, most apparent around the walls of small veins. An identical but more pronounced change was seen in the animals which had received the injection of blood directly

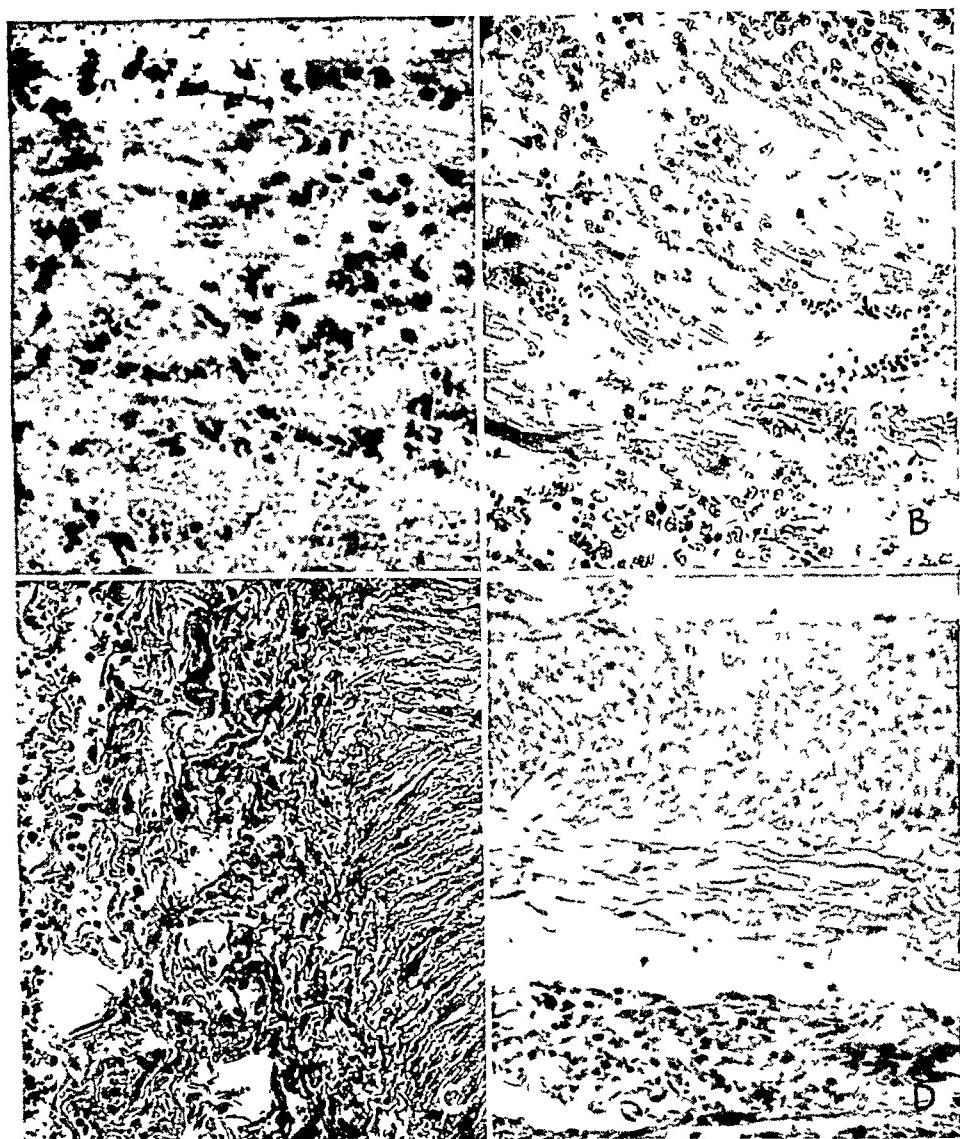


Fig. 3.—*A*, acute myocarditis with diffusely infiltrating polymorphonuclear leukocytes observed twenty-four hours after a single intravenous injection of undiluted bacterial filtrate. (Photomicrograph, ocular 5, objective 40, Zeiss.)

*B*, myocardial histiocytic hyperplasia in an animal which received two injections of rheumatic blood, one intracardially and the other, twenty-four hours later, in a peripheral vein, and was killed after forty-eight hours. (Photomicrograph, ocular 10, objective 8, Zeiss.)

*C*, lymphocytic-histiocytic proliferation occurring in the periadventitial fibrous connective tissue of a doubly ligated tract of femoral artery at the site of an intraluminal injection of rheumatic blood twenty-four hours after an intravenous injection of the same blood. (Photomicrograph, ocular 10, objective 8, Zeiss.)

*D*, lymphocytic-histiocytic hyperplasia occurring in the periadventitial fibrous connective tissue of a femoral artery of an animal killed twenty-four hours after a single local injection of rheumatic blood. (Photomicrograph, ocular 10, objective 8, Zeiss.)

into the heart (group 1). There was concurrent edema of the periaventricular fibrous connective tissue, in which occasional granulocytes could be recognized. Patchy changes in myofibrils with loss of striations and of cellular outlines were noticed in the myocardium of the animals which received an initial intracardiac injection followed twenty-four hours later by an intravenous injection of rheumatic blood (group 3). Around these degenerated areas there was moderate inflammatory reaction, mostly that of lymphocytes and histiocytes, with scattered granulocytes (fig. 3 B). This inflammatory reaction still persisted in the animals killed on the third day, while it seemed to have subsided in the animals examined on the seventh day. In the latter a proliferation of fibroblastic cells was noted.

A fundamentally identical type of tissue response, namely widespread mural and perivascular lymphocytic-histiocytic hyperplasia, accompanied by inconstant and less pronounced granulocytic infiltration, was observed in the ligated tract of femoral artery in the 3 rabbits which had received an intra-arterial injection of a few drops of rheumatic blood and twenty-four hours later an intravenous injection of the same blood (group 5) (fig. 3 C). However, both the single local injection (group 4) and the double intravenous injections one week apart (group 2) were productive only of negligible change, characterized by moderate increase of periaventricular mesenchymal cells (fig. 3 D).

#### COMMENT

The changes attending the cardiac and the arterial Shwartzman phenomenon in these animals are similar to those described by others in different organs and tissues under comparable experimental conditions. In contrast with the minimal tissue response on single administration, either local or intravenous, of the properly diluted bacterial filtrate, the striking feature of the process, both in the heart and in the femoral artery, was the hemorrhagic-necrobiotic inflammatory type of tissue reaction at the site of preparation following the intravenous injection of the filtrate. This supports the contention that the factors in the filtrate capable of producing local preparation are distinct from those responsible for inflammation and that the determining factor in the production of the phenomenon is the actual state of tissue preparedness elicited by the local administration of the filtrate.

Control experiments of Gerber<sup>12</sup> have shown that possibly particles of the medium on which the organisms are cultured, the phenolized saline solution in which the filtrate is suspended, and the autolyzed organisms unavoidably present in the filtrate may contribute in some degree to the inflammation attending local preparation. Dialyzed filtrates were used in most of the experiments to lessen the influence of these factors.

Karsner and Moritz,<sup>13</sup> also Kielanowski and Selzer,<sup>14</sup> have found evidence of vascular injury after a single local injection of the filtrate. None of the control animals in the present series which had received a

13. Karsner, H. T., and Moritz, A. R.: J. Exper. Med. 60:37, 1934.

14. Kielanowski, T., and Selzer, A.: Bull. internat. Acad. polon. d. sc. et d. lett., cl. méd., 1934, p. 417.

single dose of the diluted filtrate either locally or intravenously displayed any evidence of vascular injury, which was the predominant feature at the area of local preparation after intravenous injection of the filtrate. Vascular engorgement, thrombosis and hemorrhage were already present in animals dying two to five hours after the provocative injection. It was not ascertained whether these changes could be seen still earlier.

To explain vascular thrombosis Gerber<sup>12</sup> advocated the concept of Dietrich,<sup>15</sup> namely, "endothelial hyperactivity" initiated by the local preparatory injection and intensified by the provocative factors reaching the vessel walls by way of the blood stream. No histologic evidence of primary endothelial damage was observed in these animals, either in the small vessels within the heart or in the femoral artery, where any endothelial change, if present, could be studied in detail. Both in the prepared femoral artery and in the myocardial blood vessels significant endothelial damage was observed only in connection with extensive mural change and luminal thrombosis.

Loosening of mural structure and vascular thrombosis in a broad area account, most likely, for the hemorrhagic change accompanying the development of the phenomenon at the site of local preparation. In the majority of the animals these hemorrhagic changes were seen to be accompanied by tissue necrobiosis; in some instances, however, necrotic change alone was noticed. It affected not only the walls of the blood vessels, the cardiac myofibrils and the intervening stroma but also the granulocytes attending the inflammatory reaction. The granulocytes showed loss of structure within the nuclear confines, karyorrhexis, and karyolysis, with scattering of cellular debris. These leukocytic changes, never observed in the tissue response elicited by the single intravenous injection or by the single local injection of the filtrate, appeared to be so constant in the inflammatory reaction following the double local and intravenous injection of the filtrate as to differentiate the two types of tissue reaction still more and to point to a necrotizing factor within the tissue after preparation, accompanying or perhaps leading to the development of the phenomenon.

\* \* \*

The Shwartzman phenomenon has been considered by some as a variant of the Arthus phenomenon. The immunologic difference between the two reactions has been demonstrated by Shwartzman<sup>1</sup>; the individuality of the two phenomena has been further stressed, on morphologic grounds, by Gerber.<sup>12</sup> According to Gerber, a striking vascular

15. Dietrich, A.: Thrombose; ihre Grundlagen und ihre Bedeutung, in Aschoff, L., and others: Pathologie und Klinik, in Einzeldarstellungen, Berlin, Julius Springer, 1932, vol. 4.

response is the chief pattern of the Shwartzman phenomenon, while tissue edema and inflammatory reaction with minimal vascular damage and absence of hemorrhagic-necrobiotic change should be considered the main characteristics of the Arthus phenomenon.

The findings in the series of animals in which the effects of a non-phenomenon-producing substance were studied in the heart and in the femoral artery further point to the morphologic individuality of the Shwartzman phenomenon. The outstanding vascular injury, tissue necrosis and exudative reaction attending the classic phenomenon in the heart and in the femoral artery contrast sharply with the lymphocytic-histiocytic tissue response at the corresponding sites after injection of the non-phenomenon-producing rheumatic blood under identical procedures. The findings in the group of animals receiving rheumatic blood recall those of Seegal<sup>16</sup> in the myocardium of rabbits first submitted to repeated subcutaneous injections of foreign proteins and then given intracardial injections of the same antigens. A similar twofold reaction, histiocytic-leukocytic first and then lymphocytic, is described by Apitz<sup>17</sup> in the heart and the blood vessels of rabbits first sensitized to horse serum by previous repeated parenteral injections and then given injections of the same antigen by the intravenous route. The findings of Klinge<sup>18</sup> in the heart and the blood vessels of rabbits subjected to repeated parenteral injections of horse serum are also similar to those following injection of rheumatic blood. Klinge attributed this type of tissue response to the allergic condition created by the repeated injections of the serum. The fallacy of this conclusion, already demonstrated by a series of experiments (Ravenna<sup>19</sup>; Tedeschi<sup>20</sup>) in which identical changes were produced in a variety of experimental animals by a single parenteral injection of horse serum as well as by single injections of other foreign proteins (milk, frog blood serum), is further shown by the findings in this series of animals which had received single intravenous or intracardiac injections of rheumatic blood.

\* \* \*

Numerous attempts have been made by several investigators to reproduce carditis and angiitis in experimental animals by introducing single or repeated doses of bacterial toxins by various routes. DeVecchi,<sup>21</sup>

16. Seegal, D.; Seegal, B. C., and Jost, E. L.: J. Exper. Med. **55**:155, 1932.

17. Apitz, K.: Virchows Arch. f. path. Anat. **293**:1, 1934; J. Immunol. **29**:255, 1935.

18. Klinge, F.: Virchows Arch. f. path. Anat. **286**:344, 1932.

19. Ravenna, E.: Sulle reazioni da albumine eterogenee, Cortina d'Ampezzo. Cooperativa Anonima Poligrafica, 1934; Arch. per le sc. med. **37**:236, 1913; **44**:268, 1921.

20. Tedeschi, C. G.: Sperimentale, Arch. di biol. **88**:93, 1934.

21. DeVecchi, B.: Riforma med. **21**:16, 1905.

is accredited with having presented the first experimental pattern of toxic valvulitis, observed following repeated injections of filtrates of staphylococci, streptococci, diphtheria bacilli and typhoid bacilli in animals first subjected to valvular trauma or simultaneously treated with hypertensive adrenal gland extracts. Among the findings he emphasized the patchy necrobiotic changes, edema and swelling of the ground substance of the valve leaflets, accompanied by proliferation of histiocytes and occasional infiltration by polymorphonuclear leukocytes. A pattern of valvular lesions more or less closely fitting the description first given by DeVecchi was produced later by other investigators who repeatedly injected bacterial filtrates over a period of time (Ravenna<sup>19</sup>; Porrini<sup>22</sup>; Mencarelli<sup>23</sup>). Regardless of the type of toxin used in the experiments, degenerative change in the ground substance and proliferation of fixed connective tissue cells were found to be the prevailing patterns.

In the experiments of these investigators myocardial changes were also observed, and in those by others (Scavizzi<sup>24</sup>; Tedeschi<sup>25</sup>; Dietrich<sup>15</sup>), in which the myocardial changes were given main consideration, the predominant features of the tissue response were again patchy degeneration of myofibrils and hyperplasia of connective tissue cells, in the earliest stages mostly lymphocytes and histiocytes and later on fibroblasts, all resulting in more or less pronounced myocardial fibrosis.

If one compares these findings with those observed in the present series in the group of control animals in which single intracardiac injections of progressive dilutions of the bacterial filtrate were tested so as to reach the exact preparatory dose desired to produce the cardiac phenomenon, the more striking inflammatory type of reaction attending the tissue response in this series becomes apparent. The direct delivery of the filtrate into the heart in these experiments, compared with the devious route of introduction of the bacterial toxins (subcutaneous, intravenous) used in the experiments of others, might explain the difference in patterns; however, considering that an identical, although less severe, type of myocardial inflammatory response was seen in this series among the animals which were subjected to single intravenous injections of different dilutions of the filtrate, I think it is a more logical supposition that specific antigenic principles contained in the filtrate prepared according to the "agar washing" method were the main factor in the production of the myocardial exudative response.

22. Porrini: *Virchows Arch. f. path. Anat.* **205**:169, 1911.

23. Mencarelli, L.: *Cuore e circolaz.* **14**:273, 1930.

24. Scavizzi, P. L.: *Gior. di batteriol. e immunol.* **5**:189, 1930.

25. Tedeschi, C. G.: *Gior. di batteriol. e immunol.* **4**:1097, 1929.

Since a systematic investigation of the changes within the endocardium was not the purpose of this study, it was not made in any of the groups of animals; however, thrombosis in the parietal endocardium, with polymorphonuclear leukocytes infiltrating widely, was noticed once in the group of animals which had received a single intracardiac injection of a 1:3 dilution of the filtrate and four times among the animals submitted to the cardiac phenomenon. Gross evidence of hemorrhagic valvulitis was seen in 1 rabbit, but an inflammatory valvular response with definite granulocytes, either sparse or in small groups, was also noticed in leaflets of mitral and tricuspid valves which grossly had appeared to be intact.

\* \* \*

The considerations on the cardiac changes apply also to the changes in the femoral artery. Intraluminal thrombosis with mural hemorrhagic-necrobiotic change, acute angiitis and periangiitis, with polymorphonuclear leukocytes infiltrating widely, were consistently seen, in our rabbits, in the prepared section of femoral artery after intravenous injection of the filtrate. These vascular changes resembled to a certain extent those described by Kusama<sup>26</sup> following repeated injections of vaccines of typhoid bacilli, dysentery bacilli or streptococci. In discussing the findings of Kusama, Siegmund<sup>27</sup> concluded that they might be attributed to endothelial sensitization. It is possible that the active factors in the vaccines used by Kusama were similar in nature to those present in the bacterial filtrates employed in the present experiments; it is questionable, however, whether or not such lesions should be interpreted on the basis of an allergic condition, since both the present observations and those of Apitz<sup>17</sup> and Gerber<sup>12</sup> point to the possibility of similar changes being produced in veins, arteries and capillaries of animals receiving a single intravenous injection of the filtrate.

A vascular pathway between artery and adjacent vein has been shown with injection methods by Winternitz and LeCompte,<sup>28</sup> who succeeded in producing phleboarteritis with extension of the inflammatory process from one vascular system to the other. Examples of apparent close association between disease of artery and disease of its vein are occasionally encountered in autopsy material. The outstanding example, of course, is thromboangiitis obliterans, in which a combination of these changes is the rule. In this connection it is worth mentioning that the inflammatory process extended from artery to vein in several animals of this series.

26. Kusama, S.: Beitr. z. path. Anat. u. z. allg. Path. 55:459, 1913.

27. Siegmund, H.: Verhandl. d. deutsch. path. Gesellsch. 20:260, 1925.

28. Winternitz, M. C., and LeCompte, P. M.: Am. J. Path. 16:1, 1940.

## SUMMARY

Local tissue reactivity (Shwartzman phenomenon) was successfully produced in the hearts and femoral arteries of rabbits by the use of "agar washing" filtrates of meningococcus cultures.

Both in the heart and in the femoral artery the changes attending the development of the phenomenon were characterized by a hemorrhagic-necrobiotic, inflammatory type of tissue reaction, which contrasted with the minimal tissue response on single administration, either local or intravenous, of the properly diluted bacterial filtrate. Structural loosening in the vessel walls and vascular thrombosis were constant findings, and most likely accounted for the hemorrhagic change at the site of local preparation. In the majority of the experiments these hemorrhagic changes were seen to be accompanied by tissue necrosis; in some instances, however, necrobiotic change alone was noticed. This type of lesion, never observed in the tissue response elicited by the single local or the single intravenous injection of the properly diluted filtrate, might indicate that a necrotizing factor is present within the tissue after preparation which accompanies or perhaps leads to the development of the phenomenon.

Acute exudative endocarditis, myocarditis and angiitis, characterized by striking granulocytic infiltration, were observed among the control animals in which single intracardiac, intravenous or local arterial (femoral artery) injections of progressive dilutions of the bacterial filtrate were tested, bringing definite evidence of the possibility of eliciting acute exudative inflammatory reactions in the heart and the blood vessels by the use of bacterial toxins prepared according to the "agar washing" methods.

Duplicate experiments in which rheumatic blood was used instead of the bacterial filtrate revealed a tissue response completely different from that elicited in the demonstration of the classic phenomenon, being mainly characterized by a lymphocytic-histiocytic tissue reaction without any vascular change. This further points to the morphologic individuality of the Shwartzman phenomenon.

# CHORIONIC GONADOTROPIN IN THE DIAGNOSIS OF TESTICULAR TUMORS

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THE classification of cancers of the testis admittedly is unsatisfactory because many contain a large variety of tissues, both mesoblastic and epithelial, and some have epithelial tissues whose cells may not be identified as regards origin or level of ultimate tissue differentiation. Frequently these tumors are placed in the class of teratoma, one enigmatic term being substituted for another. A wide variety of descriptive terms have been used, and the classifications have varied greatly. Classifications for the most part have been morphologic, but some have been suggested on the basis of pathologic embryology.<sup>1</sup>

New classifications were developed when it was demonstrated that tumors might produce hormone and when gonadotrophic hormone was found in the urine of some patients with testicular tumors.<sup>2</sup>

Ferguson<sup>3</sup> advocated a classification based on quantitative determinations of the gonadotrophic hormone in the urine. He found that the quantity varied greatly and believed that a specific amount indicated the specific structural type of tumor. Biologic tests were used to make these determinations.<sup>4</sup>

Efforts to identify the type of tumor by qualitative and quantitative determinations of gonadotrophic hormone have not clarified the classification, because consideration was not given to the fact that two types of gonadotrophic hormone could be present in the urine of these patients and because the significance of each was not properly evaluated. No accurate correlation can be made between hormone determinations and specific type of tissue without consideration of these facts and of

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This study was aided by a grant from the Billings Medical Club of Chicago.

From the Henry Baird Favill Laboratory and the Department of Gynecology of St. Luke's Hospital and the Department of Gynecology of Northwestern University Medical School.

1. (a) Ewing, J.: *Surg., Gynec. & Obst.* **12**:230, 1911. (b) Christopoulos, D. G.: *Urol. & Cutan. Rev.* **44**:32, 1940. (c) Gordon, W. G.: *J. Urol.* **43**:851, 1940. (d) Melicow, W. M.: *ibid.* **44**:333, 1940.

2. (a) Hinman, F., and Powell, T.: *J. A. M. A.* **110**:188, 1938. (b) Twombly, G. H.: *Surgery* **16**:181, 1944.

3. Ferguson, R. S.: *Am. J. Cancer* **18**:269, 1933; *J. A. M. A.* **101**:1933, 1933; *J. Urol.* **31**:397, 1934.

4. Aschheim, S., and Zondek, B.: *Klin. Wchnschr.* **6**:1322, 1927.

which tissues are capable of producing the hormones. When these factors are correctly interpreted, the presence of the chorionic type of gonadotropin does indicate a specific tumor, as will be shown later.

Two cases of primary testicular tumor are reported, and a correlation between type of tumor and biologic tests is made. Both patients died from metastases, one thirteen months and the other thirty-seven months after the onset of symptoms.

#### REPORT OF CASES

CASE 1.—A white man aged 31 years entered St. Luke's Hospital on the service of Dr. Harry Culver, Oct. 13, 1944, in semicomma with complete paralysis of the right hand and arm. He died the following day. The postmortem examination was made by Dr. Edwin F. Hirsch.

The following statements were furnished by the United States Public Health Service: "The patient entered the United States Marine Hospital, Norfolk, Va., May 25, 1944, complaining of a slightly painful, gradual enlargement of the left testicle during the nine months prior to admission. Examination revealed a hard nodular mass the size of a lemon in the left testicle. The reaction to the Friedman test was positive. There was a slight increase in the red cell count and in hemoglobin. May 30 a simple left orchiectomy was done. The testicle, 4.1 by 3.0 by 2.1 cm., had attached tunics, spermatic cord and vas; it was hard and gray-white on the external surface, and on cut surfaces an irregular tumor mass had replaced most of the testicular tissue. The tumor had discrete masses of cartilage interspersed with softer, reddish yellow to gray tissues, focally hemorrhagic. It seemed confined within the testicular capsule, and a small amount of soft brown testicular tissue was present at the periphery. The tunics were thickened by a dense fibrous tissue. The cord and the vas deferens were not remarkable; the epididymis was not found.

"*Microscopic Examination.*—Sections from various portions of the tumor contained many varieties of tissue. There were masses of undifferentiated epithelial cells with distinct variation in size, shape and staining quality. The nuclei were large and had prominent nucleoli, and many cells were in mitosis. These cells had no uniformity of structure. Some masses of epithelial cells formed fairly well defined glands of various types, some suggesting renal tubules. There was also an extensive infiltration of highly undifferentiated fibrous tissue, much of it myxomatous and some of it fatty. Many cells in mitosis were in these tissues. Large masses of cartilage were distributed at random through the tumor, and there were many foci of hemorrhage and necrosis and a few with acute inflammatory exudation. The diagnosis was teratoma of testicle (mixed type, of high malignancy). The patient was transferred to the Tumor Clinic, United States Marine Hospital, Baltimore, Md., where between June 14 and July 21 he received 250 kilovolts (peak) of radiation therapy with 2,000 roentgens (r) to each of the following regions: the left side of the epigastrium, the left side of the mid-abdomen, the left groin, the posterior epigastrium and the lumbar region. Roentgenologic examination at that time revealed a number of metastatic pulmonary lesions."

Only the pertinent portions of the necropsy report are given here.

The lower margin of the right lobe of the liver extended 5 cm. below the costal arch in the right axillary line. Along the lower edge of the left lobe of

the liver was an almost continuous mass of hemorrhagic brown and gray tissue that extended for 11.5 cm. along the edge and reached into the liver 2 to 3.5 cm. It had a maximum thickness of 3 cm. There were several similar nodules on the posterior surface of the liver.

There were no adhesions between the right lung and the chest wall, but the lung had many hard nodules, some gray, others hemorrhagic and black, ranging from 1 to 3 cm. in diameter. The same conditions were observed on the left side. The unopened right lung weighed 450 Gm., and the left weighed 390 Gm. Broad surfaces made by cutting both the right and the left lung had nodules of firm and spongy tissues with recent hemorrhages.

The dura mater was a thin, gray-white membrane that stripped from the calvarium without resistance. There was little cerebrospinal fluid, and it was stained with blood. On the left side near the vertex were subpial hemorrhages along the course of the blood vessels. There was a distinct flattening of the convolutions of the brain. In the left cerebral hemisphere near the vertex, 4 cm. from the midline, was a hemorrhagic nodule in the cortex, 1.5 cm. in diameter, and another on the right side, 4 cm. from the posterior portion and 3 cm. from the midline, was 1 cm. in diameter.

The spleen weighed 310 Gm. On the lateral border 4.5 cm. from the lower pole was a nodule 1.5 cm. in diameter which projected 5 mm. above the surface. It extended 1 cm. into the splenic pulp and had an outer hemorrhagic serrated border and a yellow avascular center.

The right testis was 4 by 2.5 by 2.5 cm. On broad cut surfaces the tissue was light tan-brown and stringy. There were no changes in the epididymis. The left testicle was absent.

In the fat tissues below the pericardial sac there were nodules of hemorrhagic and black tissue.

The left kidney was 11.3 by 6 by 4.5 cm. and weighed 190 Gm. without its peripheral fat and capsule. The capsule stripped from a smooth red-brown surface except opposite a nodule of hemorrhagic tumor tissue 2.5 cm. in diameter toward the lower pole. Another hemorrhagic nodule on the anterior surface near the hilus was 2.8 cm. in diameter.

*Microscopic Examination.*—The various metastatic tumor nodules had similar tissues. In the nodules were large recent and old hemorrhages with red cells, much fibrin and scattered leukocytes. The tumor cells which had diffusely invaded the tissues were in broad and smaller masses and were of two kinds (fig. 1).

Most of the cells were like Langhans cells. They were polygonal and had large vesicular nuclei and coarsely vacuolated cytoplasm. These cells in some places varied in size and appearance approximately as did those observed in implantation sites of the human placenta. In other places the cells had huge deeply stained nuclei, and many were in mitosis. Scattered through these cell aggregates were smaller masses of syncytium in a meshwork about blood spaces. Some had engulfed erythrocytes. The syncytium in some places was spun out; in others it was solid multinucleated cell masses, and in still others it consisted of deeply stained, irregularly shaped cells with single or double nuclei.

The tunica albuginea of the right testis was dense and fibrous. The tubules had relatively thick fibrous capsules and atrophic germinal epithelium. There was little evidence of spermatogenesis. The lumens were empty, and the germinal cells had retrogressive changes. There were a few masses of interstitial cells in the loose vascular connective tissue.

*Diagnosis.*—Metastatic hemorrhagic choriocarcinoma of the lungs, the brain, the liver, the kidney, the spleen and the mediastinal lymph nodes and atrophy of the right testis.

CASE 2.—A white man aged 22 years entered St. Luke's Hospital Dec. 8, 1942, in the care of Dr. A. R. Morrow. The left testicle had never been in the scrotum; it could be felt as a small mass in the groin and for two months prior to

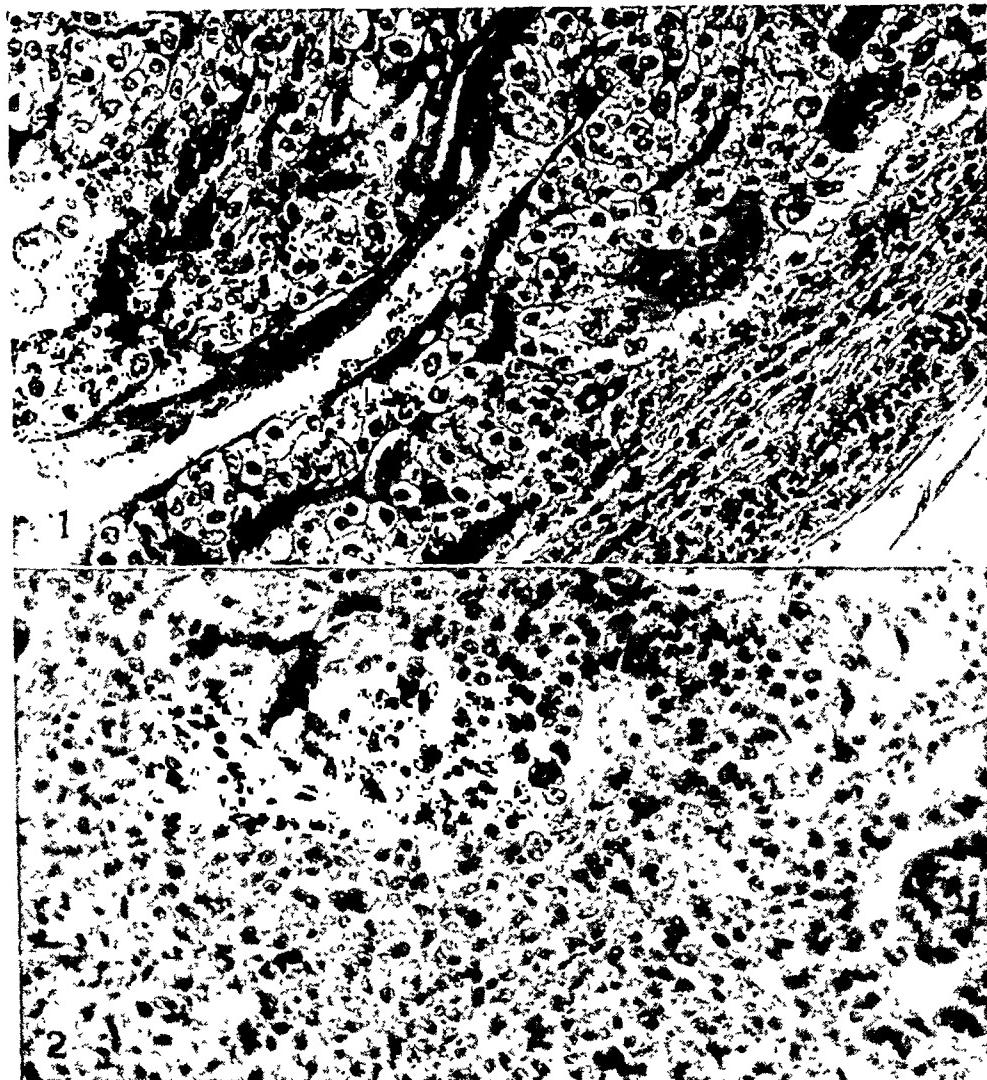


Fig. 1 (case 1).—Photomicrograph of a metastatic lesion in the lung. The cells resemble Langhans cells and syncytium of chorion.  $\times 198$ .

Fig. 2 (case 2).—Photomicrograph of a primary tumor of the testis. The tumor tissue is arranged in mosaics with some slits or lumen-like structures. There are a few deeply stained multinucleated cell masses.  $\times 198$ .

admission had increased in size. When he was examined there was a mass the size of a large egg in the left inguinal canal. The left testicle was not in the scrotum.

A simple left orchectomy was done December 10. The body of the left testicle was 4.5 by 3 by 2.5 cm. About two thirds of the substance in the body of the

testis was replaced by gray-yellow tumor tissue extending from the epididymis toward the tunics of the testis. This tumor tissue had a finely nodular appearance and was elevated above the surrounding tissue. There were small yellow regions of necrosis.

Histologic examination of the testicle revealed extensive regions of necrosis and hemorrhages. The tumor tissue consisted of large and small masses of cells arranged in mosaics and also having some tissue crevices or lumen-like structures. The cells were of medium size, with pale cytoplasm and large vesicular nuclei containing coarse chromatin granules. Many cells were in mitosis. There were a few large multinucleated giant cells (fig. 2). In some portions of the testicle the lymph channels and the blood vessels were dilated, and the lumens contained masses of tumor cells. The tissues of the body of the testicle had undergone retrogressive changes. There were many atrophic tubules with and without lining cells. In the stromal tissues of the epididymis there were several masses of tumor cells. The interstitial cells were not abundant.

The diagnosis was seminoma of the left testis with metastases in the funiculus spermaticus.

An Aschheim-Zondek test made nine days after the operation was positive. Roentgenologic examination of the chest on the seventh postoperative day (December 17) revealed no evidence of metastatic lesions in either lung field or in the ribs and no enlargement of the mediastinal glands. The patient made an uneventful recovery and was discharged from the hospital on the tenth postoperative day.

He was readmitted June 12, 1945, for cystoscopic examination and treatment of nonspecific vesiculitis, prostatitis and cystitis. An intravenous pyelogram revealed no abnormality of the kidneys or the ureters.

October 8 the patient was admitted for the third time, with an inguinal hernia on the right side. He complained of pain and swelling of the mammary glands for a period of three months, loss of 30 pounds (13.6 Kg.) in the previous three months, occasional substernal pain and hemoptysis of bright red blood on one occasion in the month prior to this admission.

There was a right indirect inguinal hernia. The breasts were enlarged, tense and tender. The lungs were normal. Roentgen examination of the lungs revealed no abnormality, and there was no change from the previous findings on Dec. 17, 1942. Roentgen examination of the lumbar spine and the pelvis revealed no abnormalities. Herniorrhaphy was done on the right side Oct. 10, 1945, and the patient was discharged October 21.

November 9 the patient was admitted for the fourth time, complaining of progressive weakness, substernal pain that was increasing in frequency and severity, loss of 50 pounds (22.7 Kg.) in six months, cough and daily hemoptysis of bright red blood. Examination revealed retraction of the supraclavicular and infra-clavicular spaces, with areas of dulness, and rales and inspiratory wheezes in both lungs. The mammary glands were enlarged.

Roentgenologic examination demonstrated both lung fields studded with multiple small nodular masses so numerous that they appeared to coalesce. An intravenous pyelogram showed the left kidney and the upper part of the ureter displaced away from the midline. The blood had 7.5 Gm. of hemoglobin per hundred cubic centimeters and 2,900,000 red blood cells and 8,650 leukocytes per cubic millimeter. Results of all other clinical and laboratory examinations were normal or negative. An Aschheim-Zondek test was not made at this time.

The clinical diagnosis was metastases of the lung from the primary seminoma of the left testicle surgically removed in 1942.

The patient died November 20. The postmortem examination was made the same day by Dr. Edwin F. Hirsch. The essentials are as follows:

Both lungs were noticeably distended and had multiple firm nodules. On cut surfaces these nodules were porous, hemorrhagic and gray-red, ranging in size to 2.5 cm. The right lung weighed 1,835 Gm. and the left 1,635 Gm.

The lymph nodes at the bifurcation of the trachea and the main bronchi were together in a mass 5.5 by 3 by 1.5 cm. On cut surfaces they had edematous red-brown lymph node tissue. In front of the esophagus just below the bifurcation were gray-red lymph nodes on both sides, the largest 5 by 2 by 1.2 cm. They were composed of firm, red-brown, blackened tissues. Around the abdominal aorta were many large lymph nodes also with spongy hemorrhagic tissue. The peri-aortic, mediastinal and mesenteric lymph nodes were similar.

Beneath the capsules of both kidneys were many small hemorrhagic nodules from pinpoint to 8 mm. in diameter. On surfaces made by hemisecting the kidneys there were small hemorrhagic lesions as large as 8 mm. scattered in the cortex.

The body of the right testis was 5.5 by 3.5 by 2.5 cm. and was composed of tan-brown stringy tissue.

Both mammary glands were enlarged. The right was 7.5 cm. in diameter and 1.5 cm. thick, and the left was 6.0 cm. in diameter and 1.5 cm. thick. At one place in the right gland was a cyst or dilated duct 4 mm. in diameter. Also in the breast tissue was a hemorrhagic tumor nodule 6 to 8 mm. in diameter.

The adrenal glands were of the usual size. The cortex of each was yellow with an abundance of lipid.

*Microscopic Examination.*—All of the metastatic lesions were similar in tissue structure. Most of the cells were in masses and resembled those of the cytotrophoblast (fig. 3). They were polygonal and had vesicular nuclei and pale, coarsely vacuolated cytoplasm. The nuclei in some were huge and darkly stained. There were also masses of syncytium with eosinophilic, vacuolated cytoplasm containing many large nuclei. In some regions the syncytium was characteristically spun out (fig. 4). There were extensive regions of hemorrhage extending into the adjacent tissues and large regions of necrosis with exudates of polymorphonuclear leukocytes, lymphocytes and large mononuclear phagocytes.

The mammary gland tissues consisted mainly of edematous fibrous stroma and dilated duct structures lined by columnar epithelium. The duct epithelium was moderately hypertrophied.

*Diagnosis.*—Metastatic choriocarcinoma of the lungs, the kidneys, the right mammary gland and the tracheobronchial, periaortic, mesenteric and mediastinal lymph glands and hypertrophy of the mammary glands.

#### COMMENT

These two tumors in their primary sites in the testicles were histologically different. In the first instance histologic identification of the various tissues led to a diagnosis of teratoma, while in the second, classification of the tissues justified only the diagnosis of seminoma. In both, however, the metastases were choriocarcinoma. When each patient was first seen with the primary testicular tumor, biologic tests for chorionic gonadotropin were positive. This suggests that the primary tumors had some similar features.

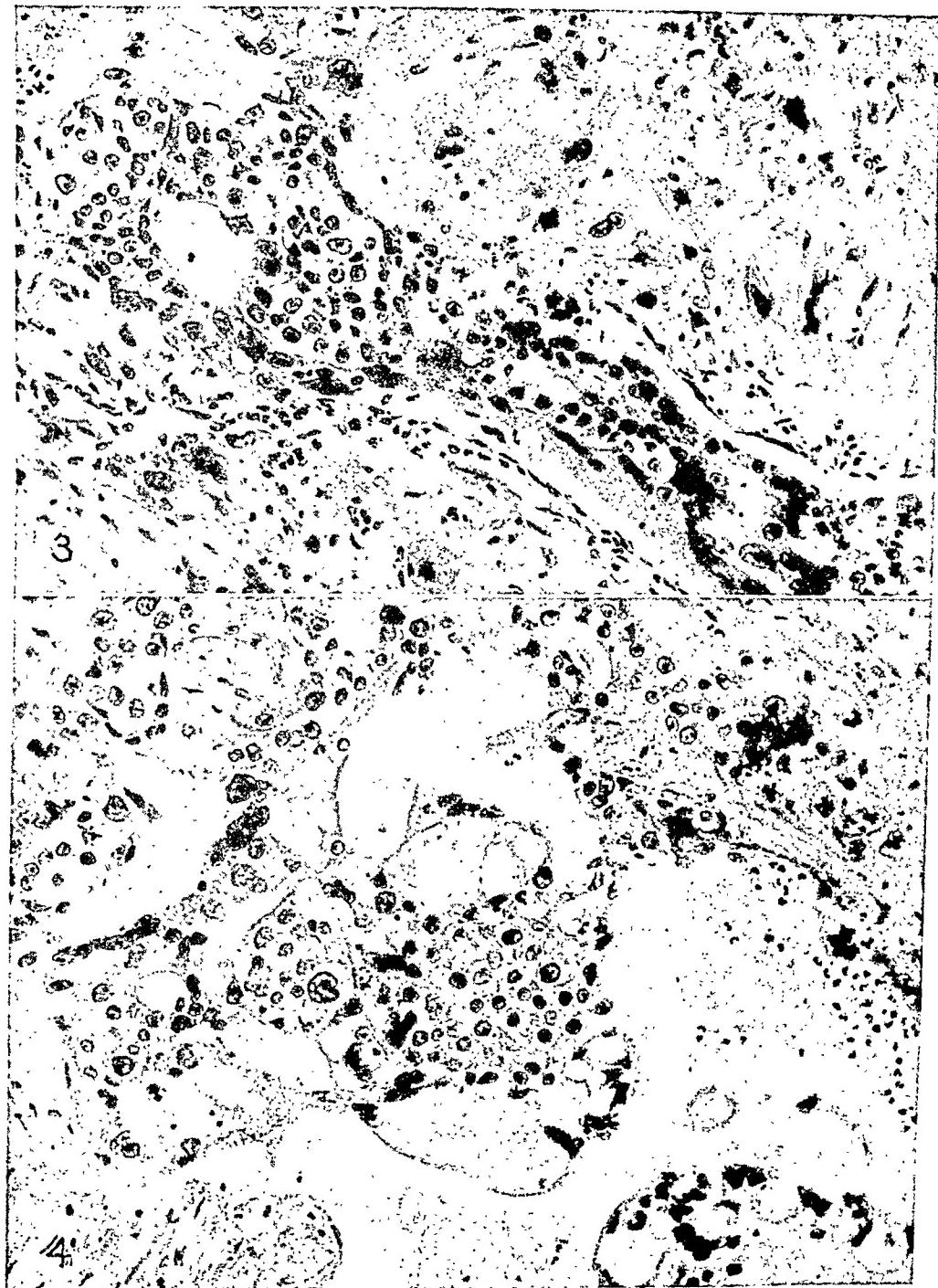


Fig. 3 (case 2).—Photomicrograph of a metastatic lesion in retroperitoneal tissues. The cells resemble Langhans cells of the chorion. There are masses of syncytium.  $\times 198$ .

Fig. 4 (case 2).—Photomicrograph of a metastatic lesion in retroperitoneal tissues showing "spun-out" syncytium.  $\times 198$ .

The biologic test described by Aschheim and Zondek<sup>4</sup> has been made in cases of various types of testicular tumors by numerous workers. It was positive in some but not all cases and it has been reported both positive and negative with the same histologic type of testicular tumor, so that its significance has been variously interpreted.<sup>5</sup> Some reports stated that the test is positive in cases of teratoma and seminoma, while others declared that it is negative in such cases. It is generally accepted that the quantitative test advocated by Ferguson<sup>3</sup> is not a reliable means of differentiating types of testicular tumors. Two distinct types of gonadotropin test reactions have been observed in cases of testicular tumor. These two reactions represent two different gonadotropic hormones. These two types of hormones and their biologic and chemical differentiation have been described by many workers.<sup>6</sup> One hormone is the chorionic type of gonadotropin that is identical with that found in the urine of pregnant women. It produces luteinization in the ovarian follicles of the test animals. The other is the castrate type that is elaborated in the pituitary gland and is found in castrates, male and female, in women past the menopause and in elderly men. This hormone produces follicle growth but not luteinization in the ovaries of the test animals.

In cases of testicular tumor distinction should be made between these two reactions. Such differentiation was not possible in earlier reports because the reactions were not at that time completely understood and accepted. Confusion has occurred in the correlation of the test and the type of tumor because of failure to differentiate properly these two test reactions. Chronic gonadotropin in the urine indicates the presence of a biologically active tissue. The presence of the castrate type of gonadotropin, on the other hand, indicates only that there is reduction or cessation of testicular function. The demonstration of this type of hormone does not in any way indicate the presence or the absence of a biologically active tumor tissue.

Further confusion has occurred as a result of a lack of a uniform histologic interpretation of a given tumor tissue and a lack of a uni-

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5. Heidrich, L.; Fels, E., and Mathias, E.: Beitr. z. klin. Chir. **150**:349, 1930. Rosenblatt, P.; Grayzel, D. M., and Lederer, M.: Am. J. Surg. **57**:94, 1942. Stevens, W. E.: J. Urol. **44**:864, 1940. Christopoulos,<sup>1b</sup> Twombly,<sup>2b</sup> Ferguson.<sup>3</sup>

6. Evans, H. M., and Simpson, M. E.: Am. J. Physiol. **89**:371, 375, 379 and 381, 1929. Engle, E. T.: J. A. M. A. **93**:276, 1929. Hamburger, C.: Acta path. et microbiol. Scandinav., 1933, supp. 17, p. 1; Compt. rend. Soc. de biol. **112**:99, 1933. Selye, H.; Collip, J. B., and Thompson, D. L.: Endocrinology **17**:494, 1933. Engle, E. T.: Am. J. Physiol. **106**:145, 1933. Leonard, S. L.: Proc. Soc. Exper. Biol. & Med. **30**:403 and 1251, 1933; Anat. Rec. **55**:26, 1933. Witschi, E., and Keck, W. N.: Proc. Soc. Exper. Biol. & Med. **32**:598, 1935. Liu, S. H., and Noble, R. L.: J. Endocrinol. **1**:7 and 15, 1939.

versally accepted nomenclature and classification of tumors. For example, some authors described embryonal carcinoma and seminoma as one and the same tumor, while others classified them as separate and distinct pathologic entities. Attempts by these authors to correlate the results of biologic tests with types of tumor could lead only to confusion.

It is apparent that in attempting a correlation the various workers have first made a diagnosis of tumor type based on certain established histologic criteria. The biologic test is then stated to be either positive or negative in association with the histologically determined type of tumor. Thus, if the tumor is diagnosed histologically as teratoma, the test is said to be positive or negative for teratoma. The correlation is not carried farther. The identity of the specific tissue in the tumor which might produce the hormone has not been adequately considered.

As a result of the facts noted, the literature contains diverse and conflicting data, the true value and the significance of the Aschheim-Zondek test has been lost, and progress toward identification, classification and understanding of testicular tumors has been retarded.

The chorionic gonadotropin is the more important of the two hormones mentioned in instances of testicular tumor. Several pertinent facts concerning the production of this hormone deserve consideration.

This hormone is produced by the cells of the fetal placenta.<sup>7</sup> Jones, Gey and Gey,<sup>8</sup> working with *in vitro* cultures of cells from the human placenta and choriocarcinoma, made assays and demonstrated the presence of this hormone after the medium had been changed several times and when the explants consisted of only new cells. They showed thus conclusively that these cells produced the chorionic gonadotropin.

In early pregnancy, at the time when the chorionic gonadotropin appears in the urine, the growth of the chorionic tissue greatly exceeds the development of the embryo proper. The tissues of the embryo itself are in such an early stage of organization that it is not conceivable that they have the ability to produce a hormone.

As the embryo develops toward its final stage and its glands and organs are more differentiated, the urinary chorionic gonadotropin titer is diminished. Most authors agree that the urinary level of this hormone reaches a peak by the sixtieth to the eighty-fourth day after the first day of the last menstrual period and then falls sharply.<sup>9</sup> At about

7. Bischoff, F.: Endocrinology **30**:525, 1942. Collip, J. B.: Canad. M. A. J. **22**:761, 1930. Ware, H. H.; Main, R. J., and Taliaferro, I.: J. A. M. A. **111**:524, 1938.

8. Jones, G. E. S.; Gey, G. O., and Gey, M. K.: Bull. Johns Hopkins Hosp. **72**:26, 1943.

9. Browne, J. S. L., and Venning, E. M.: Lancet **2**:1507, 1936. Evans, H. M.; Kohls, C. L., and Wonder, D. H.: J. A. M. A. **108**:287, 1937. Boycott, M., and Rowlands, J. W.: Brit. M. J. **1**:1097, 1938.

this time the Langhans cells, which are a part of the chorionic tissue, begin to disappear. During the remainder of the pregnancy the level is low. These facts suggest that the tissues of the fetus proper do not produce the hormone.

In some instances of abortion and tubal pregnancy in which the chorionic tissues survive and continue to grow for some time, the chorionic gonadotropin continues present in the urine although the embryo itself has been absorbed. This indicates that tissues of the embryo proper do not produce the hormone.

A well established fact is that in the human female choriocarcinoma is associated with high titers of urinary chorionic gonadotropin in the absence of any tissues of the embryo proper. It is also known that after the removal of a choriocarcinoma of the uterus the chorionic gonadotropin disappears in the absence of metastases. When metastases occur the chorionic gonadotropin reappears in the urine. In these instances no embryo is present. Accordingly the tissues of the embryo proper do not produce this hormone.

The chorionic gonadotropin in the urine of patients with testicular tumors or metastases of these is identical with the chorionic gonadotropin found in the urine of pregnant women,<sup>10</sup> and the view generally accepted is that the hormone is produced by the tumor tissues.

All the evidence, both clinical and experimental, indicates that the chorionic gonadotropin is produced by chorionic tissues, that the tissues of the embryo proper do not produce the hormone, that no other tissues are known to produce it, that when the hormone is present in instances of testicular tumor it is identical with the chorionic gonadotropin found in the urine in pregnancy and that in these instances it is produced by the tumor tissues.

It has been accepted that a tumor tissue can have a hormone-producing function, as exemplified by the granulosa cell tumor and arrhenoblastoma.<sup>11</sup> Each of these tumors may vary greatly in histologic structure. Especially in arrhenoblastoma there frequently are multiple types of tissue. The hormone produced by this tumor is not considered to originate in all the varied tissues but rather is believed to be produced by a specific type of tissue that is but a part of the tumor. In this respect arrhenoblastoma is comparable to a tumor of the testicle in which multiple tissues are present. If such a tumor should produce a hormone, and not all of them do, clinical experience and experimental evidence indicate that the hormone would arise from a specific tissue and not from all the types of tissue found in the tumor.

10. Fevold, H. L.; Fiske, V. M., and Nathanson, I. T.: *Endocrinology* **24**: 578, 1939.

11. Meyer, R.: *Am. J. Obst. & Gynec.* **22**:697, 1931.

Rather than to go far afield and believe that testicular teratoma as such, or embryonal carcinoma, or seminoma produces the chorionic gonadotropin, it would seem more consistent to believe that when the chorionic gonadotropin is present, sufficient chorionic tissue is present in the tumor to produce this hormone. A small amount of such tissue may elaborate sufficient quantities of the hormone to cause a biologic test to be positive. This is true for choriocarcinoma of the female, and it has also been observed in the case of a small testicular tumor.<sup>12</sup>

That these tissues may not be recognized in microscopic examinations is understandable. The difficulty of positively identifying chorionic cells microscopically is well known. In fact, many believe that a microscopic diagnosis of choriocarcinoma is not justified without demonstration of the chorionic gonadotropin. It is possible that chorionic tissues are present in some testicular tumors but cannot as yet be identified histologically. Another possible source of failure to diagnose chorionic tissue in a tumor is that only relatively few blocks of tissue are taken from portions of the tumor for microscopic study. Chorionic tissue might not be present in the blocks chosen for examination. Since these tissues need be only small in amount, they could be overlooked unless many more blocks were taken and more sections were made:

In the 2 instances reported here the fact that chorionic gonadotropin was demonstrated in the urine at the time the primary tumor was found indicated that the tumor contained tissue capable of producing the hormone. Since only chorionic tissue produces this hormone, chorionic tissue must have been present in the primary tumor. Microscopically, however, no chorionic tissue was identified in the sections of the tumor in the first instance, and in the second the presence of a few large cells was not considered sufficient for the diagnosis of choriocarcinoma (fig. 2). On the basis of histologic criteria a diagnosis was made in the one instance of teratoma and in the other of seminoma. Since the correlation of the results of the biologic test and types of testicular tumor was in such a confused state, the essential significance of the positive results of the test in these 2 instances was not completely evaluated. In view of the facts which have been presented, the presence of chorionic gonadotropin in the urine should have indicated that chorionic tissue was present in the primary tumor, in the one instance in association with a teratoma and in the other with a seminoma.

That this contention is true is proved by the fact that the metastases in both instances were choriocarcinoma (figs. 1, 3 and 4). For such a type of metastasis to occur, choriocarcinoma must have been present in the primary tumor. There is no reason to believe that choriocarcinoma was primary in the multiple sites in which it was found in both

12. Craver, L. F., and Stewart, F. W.: J. A. M. A. 106:1802, 1936.

patients. There is no evidence that it had an extragenital origin. Rather one is certain that in each instance it had metastasized from the surgically removed testicular tumor even though chorionic tissue was not observed in this tumor. The presence of the chorionic gonadotropin in both instances indicated the true nature of the primary tumor and the choriocarcinomatous metastases subsequently proved this to be correct.

Thus, based on the positive biologic test for chorionic gonadotropin, the accurate and complete diagnosis of the primary testicular tumor would have been teratoma with choriocarcinoma in the first instance and seminoma with choriocarcinoma in the second.

It is entirely conceivable that so-called teratoma, seminoma and other types of testicular tumors may occur with or without choriocarcinoma. If choriocarcinoma is not present, the test for chorionic gonadotropin would be negative and if present the test would be positive. The confusion that exists because the test is positive in some cases of teratoma, seminoma, etc., and negative in others would thus be explained.

#### SUMMARY

Differentiation of the chorionic gonadotropin and the castrate type of gonadotropin, both of which may be present in the urine of patients with testicular tumors or metastases of these, or both, is essential.

Chorionic gonadotropin in the urine of a patient with a testicular tumor indicates that there is present in the tumor a hormone-producing tissue. This tissue is chorionic tissue.

The chorionic tissue may not be demonstrated in the primary tumor because it may be but a small part of the growth, because the entire tumor is not examined microscopically or because it is impossible microscopically to differentiate chorionic tissue from the multiple and varied tissues of a testicular tumor.

In the 2 cases presented here a test of the urine for chorionic gonadotropin indicated that chorionic tissue was present in the primary tumor even though such tissue was not identified microscopically. That chorionic tissue was present was subsequently proved by the metastases, which in each instance were choriocarcinoma.

If chorionic gonadotropin is present in the urine of a patient with a testicular tumor, it indicates the diagnosis of choriocarcinoma even though chorionic tissue is not identified microscopically in the primary tumor.

# TOXICITY AND DETOXICATION OF CINCHOPHEN

Experimental Studies

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DESPITE a great deal of clinical, pathologic and experimental work in the past,<sup>1</sup> several important pharmacologic and toxicologic aspects of the action of cinchophen on the organism of man have remained controversial or obscure. This lack of established knowledge is particularly disconcerting because of the alleged injurious effect of cinchophen on the function and on the parenchyma of the liver and because of the resulting serious interference with the institution of effective countermeasures.

The following experiments were undertaken in an attempt to obtain additional data on the hepatotoxic action of cinchophen and on conditions possibly favoring or counteracting such an effect.

## EXPERIMENTS

*Influence of Cinchophen on the Prothrombin Time of Rats.*—Rawls<sup>2</sup> found that the incidence of the various toxic symptoms elicited by cinchophen in man could be considerably reduced by simultaneous administration of vitamin K, which, it was claimed, improved the function of the liver. On the basis of such considerations Rawls proposed the use of the prothrombin time test as a criterion of the function of the liver in persons receiving cinchophen. It seemed to be pertinent, therefore, to determine whether or not cinchophen, like salicylates, when given in excessive doses to rats kept on a diet low in vitamin K causes lengthening of the prothrombin time.

Thirty rats weighing about 125 Gm. were placed on a vitamin K-deficient diet, composed of 18 parts of casein, 8 parts of yeast, 4 parts of salt, 5 parts of cottonseed oil, 2 parts of cod liver oil and 63 parts of cerelose. The rats were allowed to eat this stock diet ad libitum during the day. During the night they were offered 5 Gm. of a corn starch-cotton seed oil mixture containing 100 mg. of cinchophen, which they usually consumed. This management was continued for six weeks. The prothrombin time was determined with Quick's method, a 12.5 per cent plasma dilution being used, before the special dietary treatment was started and at weekly intervals thereafter.

The prothrombin time, which fluctuated between 23 and 30 seconds, average 27.5 seconds, at the start of the experiment, ranged from 39 to 98 seconds, average 60.5 seconds, at the end. The rats were then placed on the stock diet, which

From the Warner Institute for Therapeutic Research.

1. Footnote deleted by the author.

2. Rawls, W. B.: New York State J. Med. 42:2021, 1942.

was fortified with 20 mg. of menadione (2-methyl-naphthoquinone) per day. After three weeks of this diet the prothrombin time had dropped to 15 to 20 seconds, average 17.5 seconds.

Cinchophen, which has a certain pharmacologic resemblance to salicylates, thus causes lengthening of the prothrombin time in rats kept on a diet low in vitamin K.

*Effect of a Low Protein, Low Methionine Diet on the Toxicity of Cinchophen.*—It is known from clinical and experimental studies that a systemic deficiency of protein, caused by insufficient intake of protein or excessive loss of protein, reduces the resistance of the liver to typical hepatotoxins, such as phosphorus and chloroform. Glynn and Himsworth<sup>3</sup> reported that such a diet, deficient in methionine, when given to rats may elicit extensive, hepatic degenerations which closely resemble the changes of acute yellow atrophy of the human liver. Inasmuch as it has been maintained that malnutrition represents an important factor favoring the development of cinchophen hepatitis in man, it was decided to feed cinchophen to rats kept on a diet with a low protein (2 per cent), low methionine content. Gelatin was used as the exclusive source of protein, as this substance contains only 0.8 per cent of methionine. The following diet was used:

Sucrose.....	25,300 Gm.
Gelatin.....	1,500
Salts.....	1,200
Corn oil.....	600
Vitamin B <sub>1</sub> .....	150 Mg.
B <sub>2</sub> .....	300
B <sub>6</sub> .....	150
Nicotinic acid.....	3 Gm.
Choline.....	6
Calcium pantothenate.....	3
Inositol.....	6
Para-aminobenzoic acid.....	3
Ascorbic acid.....	3
Cod liver oil.....	600
Total.....	30,000 Gm.

Thirty rats weighing about 150 Gm. received this diet only, while a second set of 30 rats was fed this diet supplemented with cinchophen (180 Gm. of cinchophen in 15 Kg. of the diet). Each rat was given 10 Gm. of this food daily, containing 0.06 Gm. of cinchophen.

The rats of both groups rapidly lost weight. At the end of a ten week period the average weight of the control group had dropped to 82.8 Gm. and that of the group with cinchophen added to the diet, to 69 Gm. Moreover, there were 17 deaths in the cinchophen group at the end of the experimental period against 4 deaths in the control group. The toxic effects of cinchophen became further manifest in an earlier and more marked appearance of brown incrustations of the hairs of the nape, the ears and the snout in the cinchophen series. These incrustations were present in 27 rats of the cinchophen group, in which they were in some instances of a very advanced type, while they were observed in only 13 of the control group, in which in general they were of a mild to moderate type.

These manifestations, suggesting the presence of a vitaminic deficiency, were not overcome by doubling or later tripling the daily intake of the vitamins composing the vitamin complex contained in the diet. The same failure resulted when 5 rats showing these incrustations were fed the experimental diet reenforced

3. Glynn, L. E., and Himsworth, H. P.: J. Path. & Bact. 56:297, 1944.

with calcium pantothenate, riboflavin or thiamine, respectively (100 Gm. of the diet plus 100 mg. of calcium pantothenate, 2.5 mg. of riboflavin or 26 mg. of thiamine hydrochloride).

The postmortem examinations of the rats which died during the course of the experiment and which were killed at the end of it, in both series, did not reveal any gross evidence of hepatic degeneration or necrosis. The histologic study of the livers revealed the fact that in the rats of the control series the cytoplasm of the liver cells was highly rarefied and there was mild to moderate interstitial edema, while in the cinchophen series the liver cells were of almost normal appearance. Rarefaction of the cytoplasm was rarely seen. There was some pyknosis, as well as mild to moderate interstitial edema.

The observations thus made confirm those previously reported by other investigators (Barbour and Gilman<sup>4</sup>) showing that restriction of the caloric intake and of the consumption of protein does not favor the production of diffuse and acute necrosis of the liver in animals fed cinchophen. They also demonstrate that a distinct hepatotoxic effect of the type of acute yellow atrophy is not obtained when a diet low in protein and in methionine is given in association with cinchophen.

*Effect of Cinchophen on Monkeys.*—In view of the repeated failure to produce in mice, rabbits, rats, guinea pigs, cats and dogs, by oral or parenteral administration of toxic doses of cinchophen, hepatic lesions resembling those of acute yellow atrophy of the human liver, it appeared desirable to test monkeys in this respect. Two adult Macacus rhesus monkeys, weighing 8 Kg. and 6.3 Kg., respectively, were given daily, by stomach tube, a suspension of 2 Gm. and 1.5 Gm. of cinchophen, respectively, in a 0.5 per cent tragacanth solution. One of the monkeys died after sixteen days of this management, diarrhea, weakness and somnolence having developed. The second monkey died after having lived forty-six days. He appeared to be normal during the early part of the experiment but subsequently became increasingly pale, nervous, cold and weak, and passed loose, soft stools. His appetite varied from fairly good to poor during the major portion of the experimental period. During the last days he refused food, vomited after the introduction of the cinchophen-tragacanth suspension and could scarcely be roused from his severe somnolence.

The autopsies of both monkeys revealed normal stomachs. The livers were congested and exhibited a normal structural pattern. The lungs were hyperemic and mildly edematous.

The results of this experiment show that monkeys do not seem to differ in their hepatic reactivity to cinchophen from other species, and provide thereby additional evidence for the concept that cinchophen is not a hepatotoxic chemical in the ordinary sense.

*Acute Experiments in Detoxication.*—Among the various detoxicating agents (sulfuric acid, acetic acid, choline, glycuronic acid, glycine, glutamic acid, cysteine, methionine and others) two (glycuronic acid and glycine) have been mentioned in connection with cinchophen detoxication. Lutwak-Mann<sup>5</sup> stated that rat liver does not show any ability to form conjugates of glycuronic acid with cinchophen. Quick<sup>6</sup> suggested that the detoxication of cinchophen in the body probably results from its being combined with glycine.

4. Barbour, H. G., and Gilman, A.: J. Pharmacol. & Exper. Therap. 55:400, 1935.

5. Lutwak-Mann, C.: Biochem. J. 36:706, 1942.

6. Quick, A. J.: J. A. M. A. 99:1190, 1932.

In the experiments to be reported cysteine hydrochloride, ascorbic acid and choline dihydrogen citrate were chosen as detoxicants and were given to mice which received cinchophen by mouth. From preceding experiments it was known that 25 mg. of cinchophen suspended in a mucilaginous vehicle and administered by stomach tube to a mouse weighing 25 Gm. exerts, as a rule, a lethal effect in 50 per cent of the mice. In the first experiment the following 4 groups of mice were used: 30 mice, each receiving 25 mg. of cinchophen; 30 mice, each receiving 25 mg. of cinchophen plus 25 mg. of ascorbic acid; 30 mice, each receiving 25 mg.

TABLE 1.—*Effect of Certain Natural Detoxicants of Cinchophen on the Mortality Rate of Cinchophen-Treated Mice*

Medication	Mice Dead	
	4.5 Hr.	24 Hr.
Cinchophen.....	15	15
Cinchophen + ascorbic acid.....	5	6
Cinchophen + cysteine hydrochloride.....	19	21
Cinchophen + ascorbic acid + cysteine hydrochloride.....	18	20

of cinchophen plus 25 mg. of cysteine hydrochloride; 30 mice, each receiving 25 mg. of cinchophen plus 25 mg. of ascorbic acid plus 25 mg. of cysteine hydrochloride.

The chemicals were incorporated into a 5 per cent polyvinyl alcohol solution in such a way that 1 cc. of the resulting material contained the stated dose. A syringe provided with a blunted needle was employed for the administration of the solution.

The mortality rates in the four groups four and a half and twenty-four hours after administration are given in table 1.

This experiment was repeated with a 0.5 per cent tragacanth solution being used as the suspending vehicle. A fifth group, receiving 25 mg. of choline

TABLE 2.—*Effect of Certain Detoxicants of Cinchophen on the Mortality Rate of Cinchophen-Treated Mice (Repetition of Experiment in Table 1)*

Medication	Mice Dead	
	4.5 Hr.	24 Hr.
Cinchophen.....	23	27
Cinchophen + ascorbic acid.....	18	23
Cinchophen + cysteine hydrochloride.....	30	30
Cinchophen + ascorbic acid + cysteine hydrochloride.....	30	30
Cinchophen + choline dihydrogen citrate.....	30	30

dihydrogen citrate in addition to 25 mg. of cinchophen, was added. The mortality rates are given in table 2.

As these experiments suggested that ascorbic acid might exert a limited protective action against the acute toxicity of cinchophen, a third experiment was undertaken in which increasing doses of ascorbic acid (multiple weight equivalents of the standard dose of 25 mg.) were given. The suspending agent was again a 0.5 per cent tragacanth solution. There were 30 mice in each group. The experimental conditions observed and the results obtained are evident from table 3.

It was noted that the mice receiving the higher doses of ascorbic acid appeared to be more drowsy than those fed cinchophen only.

Since the possibility existed that some of the untoward effects recorded in the last experiment might be attributable to a severe osmotic action exerted by the considerable amounts of ascorbic acid introduced, an additional experiment was devised in which this factor was excluded as much as possible by using a freshly prepared sodium ascorbate solution in place of ascorbic acid. Cinchophen was suspended in a 5 per cent polyvinyl alcohol solution for the first two groups and in a 0.5 per cent tragacanth solution for the third group. The usual dose of 25

TABLE 3.—*Mortality Rates in Three Cinchophen-Treated Groups Given Increasing Doses of Ascorbic Acid*

Medication	Mice Dead at 24 Hr.
Cinchophen (control).....	22
Cinchophen + 25 mg. ascorbic acid.....	22
Cinchophen + 50 mg. ascorbic acid.....	24
Cinchophen + 75 mg. ascorbic acid.....	22

mg. of cinchophen was given to all mice, with 30 mice in each group. The experimental conditions observed and the results obtained are presented in table 4.

From the total evidence recorded the conclusion was reached that none of various detoxicants (cysteine, ascorbic acid, choline), nor any mixture of them, exerted in acute experiments any significant and consistent protective action against a 50 per cent lethal dose of cinchophen given by mouth.

*Chronic Experiments in Detoxication.*—(a) Mice: These experiments were designed for the purpose of ascertaining the possible detoxicating power of ascorbic acid and methionine when moderately toxic doses of cinchophen were given over a period of several days or weeks, imitating in this respect more

TABLE 4.—*Mortality Rates in Two Cinchophen-Treated Groups in Which Sodium Ascorbic Was Substituted for Ascorbic Acid*

Medication	Mice Dead at 24 Hr.
Cinchophen in polyvinyl alcohol solution.....	18
Cinchophen + mg. ascorbic acid as sodium ascorbate in polyvinyl alcohol solution.....	27
Cinchophen + 25 mg. ascorbic acid as sodium ascorbate in tragacanth solution.....	30

closely the conditions existing in the therapeutic use of the drug. Methionine was selected for testing because of the recent reports crediting this amino acid with beneficial properties when used in the treatment of hepatic degenerations caused by chlorinated hydrocarbons. A 0.5 per cent tragacanth solution was used as the suspending agent for cinchophen and as the solvent for the detoxicating chemicals. The experimental conditions and the mortality rates after two weeks of daily administration of the test substances are apparent in table 5.

The autopsies performed on many of these mice, some of which died during the course of the experiment and some of which were killed at the end of it, did not reveal any significant hepatic abnormalities. The conclusion drawn from this experiment was that the toxic effects of repeated administration of cinchophen

are not unequivocally mitigated by simultaneous medication with ascorbic acid or methionine.

(b) Rats: In view of the known species-specific variations existing in metabolism and in the detoxication of poisonous chemicals associated therewith, rats were employed in a second attempt directed at determining the possible protective power of ascorbic acid in cinchophen poisoning. These rats received, mixed with 5 Gm. of their stock diet, a daily dose of 60 mg. of cinchophen. This dose is approximately the maximal amount of cinchophen which rats will consume without forced feeding. Thirty rats weighing from 130 to 180 Gm. were fed 5 to 7 Gm. of this diet daily for six weeks. The daily offering of food was then increased to 7 to 10 Gm. but after two weeks it was reduced again to 5 to 7 Gm., as the rats did not consume the higher amounts offered.

TABLE 5.—*Effect of Detoxicants of Cinchophen on the Mortality Rate of Mice Receiving Cinchophen Over Considerable Periods*

Mice	Medication	Mice Dead at 2 Weeks
30	10 mg. of cinchophen.....	18
30	10 mg. of cinchophen + 10 mg. of ascorbic acid.....	22
30	10 mg. of cinchophen + 10 mg. of methionine.....	21
30	10 mg. of cinchophen + 20 mg. of ascorbic acid.....	16
30	10 mg. of cinchophen + 30 mg. of ascorbic acid.....	15

A second group of 30 rats was given the same diet fortified with 180 mg. of ascorbic acid per 5 Gm. of food-cinchophen mixture. There were the same variations in the amounts of food offered as in the first group. The weights of the rats of both groups either remained stationary or increased mildly during the experimental period of four months. There were no significant differences in fluctuation of weight between the two groups. Three rats of the cinchophen group and 2 rats of the cinchophen-ascorbic acid group died. The surviving rats were killed at the end of the experimental period. Autopsies did not reveal any significant abnormalities of the liver or of any other internal organ in these rats.

The conclusion was drawn that rats given the maximal tolerated dose of cinchophen with their food for four months exhibit, as the only sign of an injurious action, stunting of their growth, which is mainly caused by the reduction of their food intake. This effect is not prevented by simultaneous oral administration of ascorbic acid in amounts three times as large as those of cinchophen.

(c) Dogs. An experiment similar to the previous one was conducted on 10 dogs weighing between 9 and 11 Kg. Five dogs received daily 2.5 Gm. of cinchophen wrapped into fish preserve, while a second group of 5 dogs was given 2.5 Gm. of cinchophen together with 7.5 Gm. of ascorbic acid. A few days after the start of the experiment the dogs began to vomit following the ingestion of cinchophen; therefore, the daily amount of the drug was divided into two portions, one of which was given in the morning and the other at night in the stated fashion. The incidence of these reactions was thereby decreased but not entirely eliminated. Diarrhea, sometimes of a bloody character, was an early response to the medication in both groups. The appetite of the dogs receiving cinchophen only suffered after the first week of this treatment, while in the dogs given cinchophen-ascorbic acid this reaction made its appearance after the second week.

had passed. With more prolonged feeding of the drugs, the dogs of both groups became increasingly weak and emaciated and were crouching in their cages. The dogs given cinchophen medication only died on the fifth, eighth, fifteenth, twentieth and twenty-fourth day, respectively, of treatment, while those given cinchophen plus ascorbic acid succumbed on the sixteenth, seventeenth, eighteenth, nineteenth and twenty-fourth day, respectively.

The rectal temperature was taken twice a day during the experimental period and did not reveal any significant fluctuations beyond the normal range. Blood examinations made during the first week did not show any appreciable changes in coagulation time, prothrombin time or sedimentation rate. In a few animals leukocytosis was present, accompanied by an increase of the lymphocytic elements and by moderate anemia, which was characterized by a reduction in the hemoglobin content and the number of erythrocytes. Determinations of the sugar and the uric acid content of the blood of the dogs of both groups, made on five occasions during the experimental period, revealed no significant changes in the blood sugar level and sometimes a mild lowering of the uric acid level. There were no differences in these reactions between the two groups. The postmortem examinations of the dogs revealed solitary or multiple ulcers of the stomach, usually located near the pylorus or on the pyloric ring. In a few instances ulcers were found at the lesser curvature. Some of the ulcers were large and deep. There was no demonstrable difference in the severity of these lesions between the two groups. The liver was without any gross abnormality in all animals. The histologic examination of the livers of the dogs of both groups showed moderate interstitial edema with scattered foci of degeneration of liver cells.

The observations made indicate that the addition of considerable amounts of ascorbic acid, representing threefold equivalents by weight of the toxic dose of cinchophen administered, to the normal diet did not prevent the development of the usual toxic reactions and gastric ulcers elicited by cinchophen and did not delay appreciably the occurrence of the lethal response. The absence of any evidence of acute yellow atrophy of the liver in any of the dogs confirms similar findings previously made by other investigators.

#### COMMENT

The experiments reported show that cinchophen when given in excessive doses to rats kept on a diet low in vitamin K causes lengthening of the prothrombin time, and that, on the other hand, no such effect is obtainable in dogs fed a normal diet. Cinchophen thus behaves in this respect like the salicylates. It is improbable, therefore, that patients on a normal diet and given therapeutic doses of cinchophen by the generally adopted intermittent method of treatment will ever exhibit any lengthening of prothrombin time unless there develops severe hepatic degeneration.

The observations made in connection with the experiments on the influence of a low protein, low methionine diet on the toxicity of cinchophen, as well as those associated with the various detoxication experiments, provide additional weighty evidence militating against the

concept that cinchophen is a hepatotoxic substance in the ordinary sense of the word. None of the animals belonging to several species (mice, rats, dogs, monkeys) exhibited hepatic lesions comparable in any way to those present in acute yellow atrophy of the liver in man. While it is possible that such unfortunate and rare therapeutic accidents seen after the administration of cinchophen may have a chemoallergic origin, this is by no means the only possibility. The evidence obtained in the present experiments indicates that malnutrition such as that caused by an insufficient intake of protein and particularly of methionine does not favor the development of severe and diffuse degenerative and necrotizing changes in the livers of animals given single and repeated toxic doses of cinchophen.

Attempts to protect animals given toxic or lethal single and repeated doses of cinchophen by the simultaneous administration of several natural detoxicating agents (ascorbic acid, cysteine hydrochloride, choline dihydrogen citrate, methionine) in doses representing one to threefold equivalents in weight of the cinchophen dose given failed completely. While the possibility remains that such agents may be effective in man, it is regarded as remote, especially as to the prevention of potential hepatic injury.

Finally, attention may be called to an observation made during the course of the experiments with a diet low in protein and methionine. It was found that in many of the rats the hairs of the nape, the ears and the snout showed porphyrin incrustations which were more marked and occurred earlier and more generally in the rats receiving the special diet together with cinchophen than in those fed the diet only. It was noted that these manifestations, usually found in connection with certain vitamin deficiencies (riboflavin, pantothenic acid), were not obviated when large amounts of these vitamins were added to the diet. Mention may be made in this connection of observations made by Bessey and Wolbach,<sup>7</sup> who found in rats kept on a diet deficient in lysine or tryptophan corneal lesions similar to those in animals with riboflavin deficiency, while Maun, Cahill and Davis<sup>8</sup> noted corresponding ocular changes in rats kept on a diet deficient in leucine. These findings suggest that there may exist a connection between deficiencies of certain vitamins and certain amino acids or their metabolic utilization, on one hand, and abnormalities of the prophyrin metabolism and corneal vascularization and ulceration, on the other.

7. Bessey, O. A., and Wolbach, S. B.: J. Exper. Med. 69:1, 1939.

8. Maun, M. E.; Cahill, W. M., and Davis, R. M.: Arch. Path. 40:173, 1945.

## SUMMARY

Cinchophen given to rats kept on a diet low in vitamin K lengthens the prothrombin time, but it does not have this effect when administered to dogs fed a normal diet.

Rats receiving a diet low in protein and methionine and given, in addition, toxic doses of cinchophen do not show diffuse and severe degeneration of the liver, but exhibit porphyrin incrustations of the hairs of the nape, the ears and the snout similar to those observed in riboflavin and pantothenic acid deficiency.

Monkeys given toxic doses of cinchophen by mouth do not have as a result acute yellow atrophy of the liver or gastric ulcers.

Mice, rats and dogs given single and repeated toxic and lethal doses of cinchophen by mouth and simultaneously various detoxicants (ascorbic acid, cysteine, methionine, choline) do not show any appreciable mitigation of the toxic symptoms or delay of or protection against the lethal effect.

## HUMAN STRONGYLOIDIASIS WITH INTERNAL AUTOINFECTION

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DESPITE the frequency of strongyloidiasis in many tropical and subtropical countries,<sup>1</sup> only little can be found in the literature concerning the histologic aspects of this disease as it occurs in man and even less concerning the internal autoinfection with *Strongyloides stercoralis*. Thus Faust and De Groat,<sup>2</sup> reporting internal autoinfection in a case of human strongyloidiasis, list not more than four papers containing reports of 5 analogous cases. This scarcity of case reports and exact descriptions of the histologic changes can be easily explained by the generally benign course of the disease, by the difficulty of finding the parasites at gross examination and by the fact that routine microscopic examination of the intestines is only rarely performed and its value often rendered illusory by extensive autolysis and putrefaction.

It can therefore not be contended that the histologic picture of human strongyloidiasis with or without internal autoinfection is well established, and thus the description of an additional case seems worth while, especially since the conditions under which the autopsy was performed were very favorable for the subsequent histologic examination.

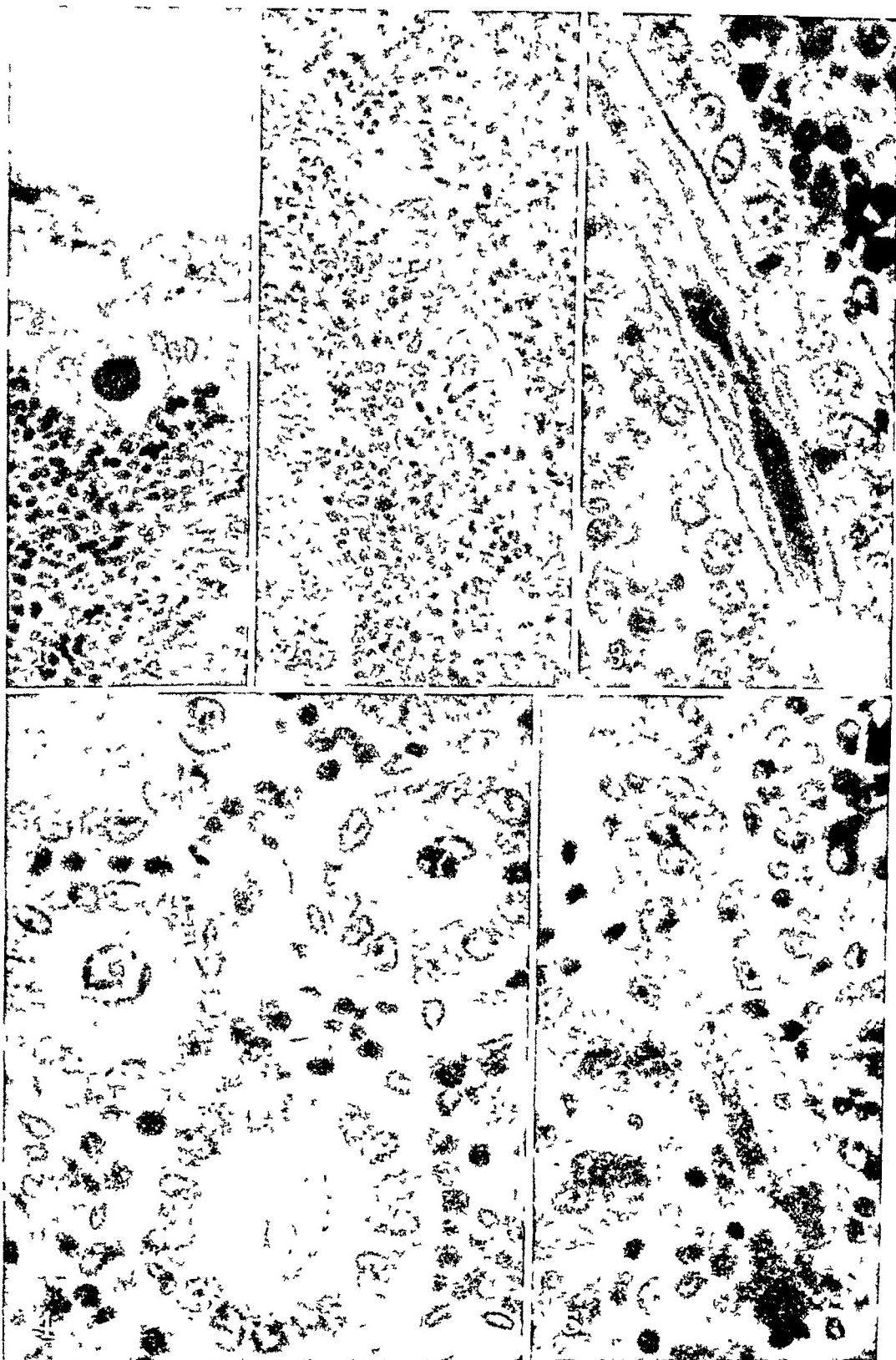
### REPORT OF A CASE

A Negro 46 years old was admitted in a bad condition to the hospital for patients with mental disorders. Clinically he presented the classic picture of dementia paralytica with megalomania. The Nonne-Apelt and Pandy reactions of the cerebrospinal fluid were positive, and the colloidal gold curve was of the paretic type. The glucose content was 110 mg. per hundred cubic centimeters. A few weeks before his death the patient became bedridden. During the last two days he had frequent thin, evil-smelling stools, macroscopically without blood and mucus. He died nine months after admission.

Autopsy was performed forty minutes after death. The internal organs were atrophic. The mucous membranes of the colon and the ileum were somewhat

1. (a) Brumpt, E.: *Précis de parasitologie*, ed. 5, Paris, Masson & Cie, 1936, p. 889. (b) Faust, E. C.: *Human Helminthology*, ed. 2, Philadelphia, Lea & Febiger, 1939, p. 391. (c) Belding, D. L.: *Textbook of Clinical Parasitology*, New York, D. Appleton-Century Company, Inc., 1942, p. 271. (d) Strong, R. P.: *Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases*, ed. 7, Philadelphia, The Blakiston Company, 1944, p. 1278.

2. Faust, E. C., and De Groat, A.: *Am. J. Trop. Med.* **20**:359, 1940.



Figures 1 to 5  
*(See legend on opposite page)*

swollen and hyperemic; no ulcers were observed. There was some thickening of the wall of the duodenum. The mesenteric lymph nodes were swollen. Examination of the brain was impossible.

*Microscopic Examination.*—The duodenum was fixed in Bouin's fluid; from the colon and other organs blocks were fixed either in Orth's or in Bouin's fluid. Péterfi's methyl benzoate-celloidin method for paraffin embedding was used; for the blocks fixed in Orth's fluid the celloidin (a concentrated preparation of pyroxylin) in the methylbenzoate medium was increased to 4 per cent. The sections were stained with hematoxylin-azophloxine, orange-eosin-toluidine blue, phloxine-methylene blue, gallamine blue, azocarmine-aniline blue, phosphotungstic acid-hematoxylin, Gram's stain and mucicarmine.

(a) Duodenum: The adult worms were not numerous. They were found partly in the stroma of the villi or in the intervillar spaces. Rarely they were lying in the deeper parts of the crypts of Lieberkühn. The cuticula of the worms showed fine cross striation, which was especially well visible with Gram's stain, as this stained the cuticula pale violet. The cross striation consisted of alternating lighter and darker staining bands, the latter also showing faint longitudinal striation. Where the adult worms were lying in the stroma of the villi there was surprisingly little reaction. Only in one place a small giant cell was observed in close contact with a worm, and the stroma of the villi containing adult worms was infiltrated by plasma cells, as was also the stroma of the noninfected villi. Eosinophilic leukocytes were seen in moderate numbers, but there was no pronounced eosinophilia. The adult worms were never surrounded by epithelial sheaths, aside of course from those parts of the worms which occupied an intraepithelial position.

The eggs and the earlier developmental stages of the larvae were found exclusively in the epithelium. They were often lying in groups. They were not confined to the epithelium of the crypts but were frequently observed in the epithelium of the villi, sometimes even on the top of a villus. The eggs or the younger larval stages were completely surrounded by epithelial cells, which sometimes were greatly thinned and contained flattened nuclei but remained recognizable as such.

The older larvae seemed to have migrated from their intraepithelial position into the crypts or into the intestinal lumen, where they were found in great numbers. When in the lumen, they were never surrounded by epithelial sheaths; it should, however, be noted that during the final stages of the migration from the "epithelial chamber" the epithelial cells could be greatly drawn out and thinned and still adhered to worms that were already lying in the lumen. Intraepithelial cavities through which adult worms had migrated, or which had con-

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#### EXPLANATION OF FIGURES 1 TO 5

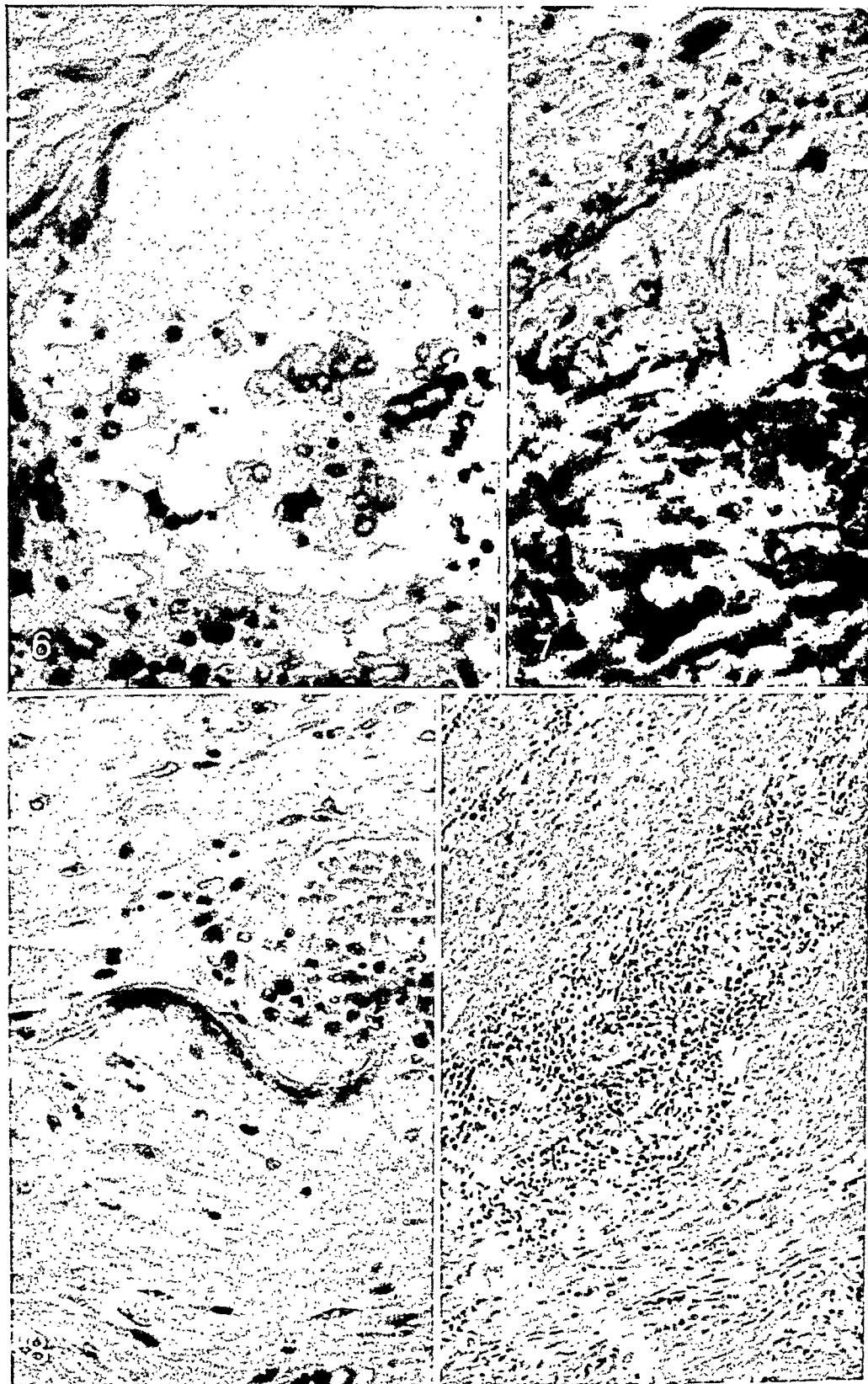
Fig. 1.—Tip of a duodenal villus with an egg of *Strongyloides stercoralis* in an intraepithelial position. Hematoxylin-azophloxine;  $\times 315$ .

Fig. 2.—Acute inflammation of the duodenum. The epithelium contains many leukocytes, but there is no desquamation. Hematoxylin-azophloxine;  $\times 315$ .

Fig. 3.—Larva in the lumen of a crypt of Lieberkühn in the colon. Phosphotungstic acid-hematoxylin;  $\times 645$ .

Fig. 4.—Cross sections of crypts of the colon containing larvae. Phosphotungstic acid-hematoxylin;  $\times 645$ .

Fig. 5.—Larva emerging from a crypt in the colon. The epithelium is hyperplastic and shows several mitotic divisions. Hematoxylin-azophloxine;  $\times 500$ .



Figures 6 to 9  
*(See legend on opposite page)*

tained larvae and were now empty, were relatively rare; they contained sometimes large gram-positive and gram-negative bacteria and gram-positive cocci, whose nature could not be determined more exactly.

The epithelium of the villi was mostly intact, and in the proximal parts of the duodenum small islets of gastric epithelium could be recognized. Mitotic divisions of epithelial cells were not confined to the crypts but were seen also in the tips of the villi. Sometimes the impression was gained that in places the epithelium had become multilayered.

In many places, in the crypts and near or on the tips of the villi there were marked inflammatory changes, only rarely combined with fibrinoid necrosis of the tips of the villi. When there was inflammation in the deeper portions of the mucosa, there were nearly always larvae, together with bacteria and leukocytes, in the lumens of the crypts. Many leukocytes were seen migrating through the epithelium, which on the tips of the villi had sometimes degenerated or become necrotic. There was neither desquamation of epithelial cells nor fibrosis of the mucous membrane resulting from previous inflammation.

Brunner's glands were strongly developed and formed large accumulations of glandular tissue. Between the tubules of the glands there were a few plasma cells and eosinophilic leukocytes. In a few instances embryonated eggs were found in the epithelium or a larva in the lumen of one of these glands. There was a moderate number of mitotic divisions in the glandular epithelium. In many places the muscularis mucosae was poorly developed or completely absent.

(b) Colon: The surface epithelium was practically everywhere intact and only in a few places could small ulcers be seen microscopically. The epithelium of the crypts showed numerous mitotic divisions; it contained a moderate number of enterochromaffine cells and an occasional Paneth cell.

The larvae, the majority transformed into filariform larvae, invaded the intestinal wall by first entering the lumens of the crypts of Lieberkühn, as could be demonstrated in longitudinal and cross sections of the crypts, and by subsequently migrating through the epithelium, especially of the deeper portions of the crypts, with invasion of the stroma. It should be noted that this happened quite independently of the microscopically small ulcers. The impression was gained that during this migration the few rhabditiform larvae which had entered the crypts also transformed into filariform larvae. Where the larvae migrated through the epithelium, the epithelial cells appeared to be very large, with nuclei containing large nucleoli; in one place there were three or four mitoses just adjacent to the place of penetration. There were a few cystic dilated crypts containing leukocytes; from one of these crypts a larva was seen penetrating into the mucosal stroma.

The more superficial part of the mucosal stroma was in general densely infiltrated by plasma cells and by varying numbers of eosinophilic leukocytes.

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#### EXPLANATION OF FIGURES 6 TO 9

Fig. 6.—Dilated lymph vessel in the submucosa of the colon. Large histiocytes surround part of a necrotic larva. Hematoxylin-azophloxine;  $\times 315$ .

Fig. 7.—Larvae in partially obliterated lymph vessel of the colon. Azan stain;  $\times 315$ .

Fig. 8.—Larva in the submucosa of the colon; the worm is partially degenerated. There is an accumulation of eosinophilic leukocytes. Hematoxylin-azophloxine;  $\times 315$ .

Fig. 9.—Granulomatous inflammation in the muscular coats of the colon. Hematoxylin-azophloxine;  $\times 150$ .

In the deeper layers, where often many larvae were present, the reaction provoked by the worms varied between wide limits. Giant cells were present in moderate numbers; they were of the smaller type; often worms, which by the strong eosinophilia and smudginess of their cuticula have perhaps to be considered as degenerating, were surrounded by large histiocytes, whereas a few others did not seem to have provoked any definite reaction. Some larvae were lying in small abscesses. Eosinophilic leukocytes were present in small numbers, sometimes lying together in small groups.

In the submucosa the lymphatics showed important but varying alterations. They all presented a more or less marked dilatation, especially the parts of the lymphatics which were lying close to the muscularis mucosae. Many contained large numbers of large and small lymphocytes and degenerating neutrophilic leukocytes; in others many eosinophilic leukocytes or erythrocytes were found. The presence of the larvae in the lumens of the lymphatics provoked reactions which varied in extent and type. In a few instances the vessels were filled with a fibrinoid substance, atypical giant cells, few epithelioid cells and some degenerating leukocytes, between which the remains of a dead larva could be detected only with difficulty. In other lymphatics intact larvae were observed which had provoked no reaction at all. Most frequently, however, the lymphatics in which there were larvae contained numerous large, rounded, sometimes finely vacuolated phagocytes with rounded to oval nuclei containing dustlike chromatin and one or two small but distinct nucleoli. Their protoplasm was faintly basophilic and contained sometimes phagocytosed cell rests. These cells occurred as isolated elements or were arranged in groups or solid sheets; they were accompanied by lymphocytes of different size and by a few leukocytes. They surrounded and sometimes completely encapsulated the larvae present in the lymphatics. In other vessels a kind of granulation tissue, composed of large cells which could be best described as forms intermediate between histiocytes and fibrocytes, with branching fibers, staining with aniline blue, between them, had encapsulated the worms and blocked the vessels. In this granulation tissue giant cells or eosinophilic leukocytes were only rarely seen. Sometimes a fibrinoid substance surrounded the worms or was found between the cells. There were no mitoses.

In a few instances larvae were found in small veins.

It was difficult to determine whether the larvae were still intact at the moment of fixation. Often it was observed that the cuticula, which in probably intact larvae was a thin membrane, staining red with phloxine-methylene blue or with orange-eosin-toluidine blue, became irregularly thickened and smudgy in larvae encapsulated by histiocytes, although the parenchymal cells of the worms did not show any changes. When the nuclei of these cells showed chromatolysis and the worm stained diffusely with eosin, it could of course be considered as dead.

In the connective tissue of the submucosa a few intact larvae were found. The majority, however, appeared to have been damaged and had provoked severe reactions. Their cuticulas showed the irregular thickening just mentioned, and often short bands of a fibrinoid substance seemed to radiate from the cuticular surface. Some larvae were surrounded by neutrophilic polymorphonuclear leukocytes; others, however, were surrounded by pale-staining histiocytes with relatively large nuclei, which often formed small giant cells by coalescence, by lymphocytes and plasma cells and especially by large numbers of eosinophilic leukocytes. Together these cells formed what can be designated as eosinophilic granulomas. It also happened that a part of a larva appeared to be intact and the rest degenerating and surrounded by leukocytes or histiocytes or both.

When there were a great number of worms, the submucosa was sometimes studded with small eosinophilic granulomas. Many larvae had penetrated into the muscular coats and into the subserous connective tissue. There were many worms in the intermuscular connective tissue septums, containing the blood vessels and nerves. These septums had been much broadened, and the connective tissue had been replaced to a large extent by granulation tissue containing large numbers of eosinophilic leukocytes. Other larvae had penetrated between the muscle fibers, provoking acute or granulomatous reactions in the edematous muscular tissue. Here several worms showed far advanced disintegration, their bodies being diffusely eosinophilic. In the subserosa and in the mesocolon and the meso-sigmoid there were many granulomas, with small giant cells containing few but

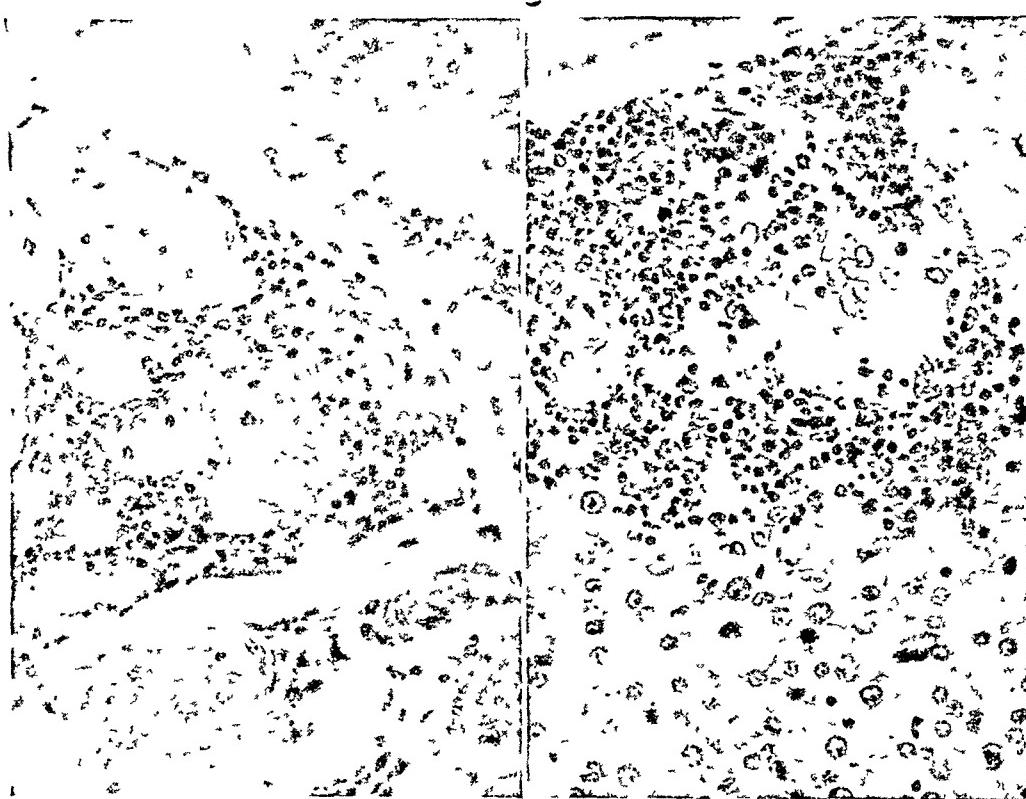


Fig. 10.—Mesocolon showing an eosinophilic granuloma with pale-staining histiocytes, containing a larva, which is seen in cross section. Hematoxylin-azophloxine;  $\times 315$ .

Fig. 11.—Subcapsular eosinophilic granuloma in the liver. Hematoxylin-azophloxine;  $\times 315$ .

large nuclei and pale-staining protoplasm and many eosinophilic leukocytes, enclosing larvae. However, there were also intact larvae lying free between the connective tissue fibers or sometimes in lymph vessels, without having provoked any reaction.

(c) Small Intestine: One block of small intestine was examined. There were a few isolated eggs in the epithelium of the villi; the mucosal stroma was hyperemic and infiltrated by plasma cells. Pathologic changes of the type which

occurred in the colon were observed here, though on a lesser scale. However, the number of intact larvae present in the submucosa without having provoked any reaction was much greater.

(d) Mesenteric Lymph Nodes: The cortical follicles were atrophic; the medullary cords were well developed; they contained many large lymphocytes; these cells occurred also in great numbers in the intermediate sinuses. There was definite hypereosinophilia, the eosinophilic leukocytes being irregularly scattered through the nodes. In one node a partially disintegrated filariform larva was seen; one part stained diffusely with eosin; the other part contained well preserved nuclei. The worm was surrounded by a small number of eosinophilic and neutrophilic leukocytes and a few histiocytes.

(e) Liver: The centers of the small lobuli disclosed hyperemia and fatty infiltration, as well as atrophy and pigmentation of the liver cells. Mitoses in liver cells were fairly numerous. There were almost no eosinophilic leukocytes in the sinusoids.

Filariform larvae were observed just below the capsule and sometimes deep in the liver in the periportal spaces. All larvae were enclosed in small eosinophilic granulomas.

(f) Stomach: Only one block, taken from the border region of corpus and antrum, was examined. It showed some chronic inflammation with marked proliferation of the epithelium.

(g) Pancreas: There was marked atrophy of the acinous tissue and of the islets. Several small intralobular ducts were dilated and contained inspissated secretion.

(h) Other organs: The heart and the adrenal glands did not show anything worth mentioning.

#### COMMENT

The case described presents several interesting features. In the first place the almost complete absence of ulceration in the intestine should be noted. The few microscopic ulcers that were seen were probably caused by the presence of larvae in the mucous membrane and represented small abscesses that had opened into the intestinal lumen. The invasion of the intestinal wall was quite independent of this ulceration. It could be demonstrated that the larvae entered the crypts of Lieberkühn and from there invaded the mucous membrane. Before or during this invasion most larvae transformed into filariform larvae. In the mucous membrane they provoked inflammatory reactions of different type and intensity. The larvae seen in the colon were probably coming from the small intestine, especially from the duodenum, the only place where adult worms and eggs in large numbers were found.

After penetrating into the submucosa and into the other layers of the intestinal wall, the larvae provoked the formation of numerous small granulomas composed of histiocytes, small giant cells, plasma cells and large numbers of eosinophilic leukocytes. These eosinophilic granulomas were also found in the mesocolon and in the liver. Sometimes more acute reactions were observed, especially in the muscular coats.

The alterations of the lymphatics, especially in the submucosa, were conspicuous. Apart from dilatation of these vessels and the presence in the lumens of many large lymphocytes and eosinophilic leukocytes, there was granulomatous endolymphangitis, provoked by the presence of the larvae in the lumens. This granulomatous inflammation led to encapsulation of the larvae and often to more or less complete obliteration of the involved lumens with consequent dilatation of the nonobliterated peripheral parts of the vessels. In contrast to filarial endolymphangitis, in which oval or more elongated epithelioid cells predominate,<sup>3</sup> here large, rounded, finely vacuolated histiocytes were more frequent; in what must be considered as more advanced stages, when fibers staining with aniline blue developed between the cells, the difference from filarial endolymphangitis became less outspoken, though, in contrast to filariasis, there were always worms inside the granulomas.

In several instances intact larvae were found in venules of the submucosa or in intact lymph vessels. In this way the lymph nodes and the periportal spaces in the liver probably were infected. The larvae found just below the capsule of the liver may perhaps have migrated from the peritoneal cavity, after migrating through the intestinal wall, though this cannot be proved.

It is not difficult to understand that the inflammation of the wall of the bowel, together with the alterations of the lymphatics, may have caused marked functional disturbances; unfortunately the patient was not closely observed and apart from the diarrhea nothing special was noted.

Disintegration of the larvae was observed; it was difficult to detect the early stages of this process and to determine whether or not a worm was still intact. Leukocytic invasion of the larvae was only rarely seen, though it occurs in later stages of disintegration, as was observed in another case.

In the liver and in the lymph nodes only slight changes were noticed. In the liver the worms were lying inside granulomas. I do not think that the fact that numerous mitoses were seen in the liver cells should be attributed to the presence of larvae in that organ.

In the lymph nodes no granulomas were found; the only larva found here was surrounded by neutrophilic and eosinophilic leukocytes and a few histiocytes.

Faust and De Groat<sup>2</sup> observed in their case that once the larvae had broken through the barrier of the muscularis mucosae the damage to the tissues was slight, and no attempt other than moderate eosinophilic infiltration was made to stay the migration of the filariform larvae. This is in marked contrast to my case, in which there were many eosinophilic

3. Hartz, P. H.: Am. J. Clin. Path. 14:34, 1944.

granulomas and conspicuous granulomatous endolymphangitis. This great difference in cellular reaction against the same parasite in 2 persons must perhaps be explained by difference in reactivity of the hosts; the immunity which can be obtained experimentally against *Strongyloides* points in this direction.<sup>4</sup>

In the duodenum the eggs were found exclusively in the epithelium and never in the stroma. There was proliferation of the epithelial cells, proved by the occurrence of mitotic divisions, even on the tips of the villi. No desquamation of epithelial cells was observed, and it is believed that the epithelial desquamation described by Strong<sup>1d</sup> in the duodenum in strongyloidiasis must be attributed to postmortem changes.

The eggs and the young larvae probably exert a stimulating effect on the epithelium, thus causing proliferation. In this way, also by the pressure exerted by the growing larvae, the small "incubation chambers" with partially flattened cells are formed, from which later the rhabditiform larvae escape. I cannot agree with Faust<sup>5</sup> when he considers this a defensive measure of the body acting against the parasites. Such active encapsulation of parasites by true epithelium would be an exceptional reaction from the standpoint of mammalian pathology, and in my opinion the opposite is the case; the larvae are protected by the epithelium during their development.

It is logical that when an adult worm penetrates through the epithelium the epithelial cells are pushed aside and compressed, just as can be observed on a larger scale in a freshly fixed colon containing trichocephalus. To designate such compressed epithelium as an adventitious sheath is certainly an exaggeration. It is also possible that these sheaths, as depicted by Faust,<sup>5</sup> are artefacts produced by ungentle handling of the tissues during autopsy or preparation. As several of Faust's photomicrographs<sup>6</sup> show extensive damage of the tissues, this possibility cannot be excluded.

In several places there was acute inflammation of the duodenum of a type not commonly observed, which was probably caused indirectly by the presence of the larvae and adult worms, since several cavities formed by the worms or eggs contained large numbers of micro-organisms.

That a few eggs and larvae were found in Brunner's glands can be explained by the fact that in duodenums with strongly developed or hyperplastic Brunner's glands the muscularis mucosae, which otherwise seems to serve as an effective barrier against the penetration of the

4. Culbertson, J. T.: *Immunity Against Animal Parasites*, New York, Columbia University Press, 1941, p. 211.

5. Faust, E. C.: *Arch. Path.* **19**:769, 1935.

6. Faust,<sup>5</sup> figs. 5, 8, 12A and 15.

worms, may be partially absent. It is not sure whether or not this hyperplasia and the mitotic activity of Brunner's glands must be attributed to the presence of the worms, as they can be observed in different circumstances.<sup>7</sup>

#### SUMMARY

A Negro who died of dementia paralytica was found to be infected with *Strongyloides stercoralis*. The adult worms were found exclusively in the duodenum. The larvae invaded the small intestine and especially the colon by entering the crypts of Lieberkühn and penetrating through the epithelium. The invasion was independent of ulceration. The larvae generally were transformed into the filariform type. After migrating through the mucous membrane, where they provoked inflammatory reactions of different type and intensity, they invaded the deeper layers of the intestinal wall, where they caused the formation of numerous eosinophilic granulomas which encapsulated the larvae and which were also found in the mesocolon and in the liver. A few intact larvae were noted in lymph vessels, in small veins and in the connective tissue of the submucosa and the subserosa of the small intestine and colon. In many lymphatics the larvae provoked granulomatous endolymphangitis often with occlusion of the lumens and dilatation of the peripheral parts of the vessels. It could be distinguished from filarial lymphangitis. In the mesenteric lymph nodes there was moderate diffuse eosinophilia but no granulomatous reaction; the granulomatous reaction was present again in the liver. It must be considered probable that the extensive alterations of the colon caused important functional disturbances.

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7. Hartz, P. H., and van der Sar, A.: Am. J. Path. 20:931, 1944.

## TROPICAL ULCER IN GUATEMALA

Pathologic, Bacteriologic, Mycologic and Clinical Aspects

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MEDICAL CORPS, ARMY OF THE UNITED STATES  
AND

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GUATEMALA

TROPICAL ulcer affects great numbers of the population in Central American countries. Because of the chronicity of the lesion, the disease is responsible for a large percentage of the hospitalizations in those countries. The plan of procedure devised for this study encompassed the selection of patients with early, "untreated" lesions as far as possible, in order to avoid interference with subsequent pathologic and microbial studies. It was soon found, however, that most patients had attempted some form of self medication. A few with totally untreated lesions were found, and the remainder were selected as far as possible from those whose therapy appeared least likely to interfere with our studies. Observations on a total of 24 patients are presented here. The history of the lesion was secured in the patient's words. In the physical examination the appearance of the ulcer was recorded, the skin was surveyed for abnormalities, a search was made for predisposing factors such as varicose veins, arteriosclerosis, diabetes mellitus or the sickling trait of erythrocytes, the oral temperatures, the pulse rates and the respiratory rates were recorded, the thorax and the abdomen were observed by the usual methods, and the spleen and the superficial lymphatic system were tested by palpation, particular attention being paid to the lymph nodes regional to the ulcer. Specimens of the urine and the stools were examined, serologic tests for syphilis and complete blood counts were made and sedimentation rates observed for most of the patients. A dietary history was obtained from 23 of the 24 patients, who were asked about the foods taken at their usual meals, and the data were recorded qualitatively. If one considers the rather monotonous diets of our patients, the method used was of fair accuracy. In each instance the lesion was then photographed. Whenever possible with those selected for biopsy, ulcer medication, if any, was discontinued for a few days prior to the

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study, and the ulcer was covered with sterile gauze squares soaked in isotonic solution of sodium chloride. Iodine-alcohol disinfection of the cutaneous borders before biopsy was followed by intracutaneous injection of a 1 per cent solution of procaine hydrochloride sufficiently distal to the edge of the ulcer to avoid tissue artefacts on subsequent microsection. Immediately prior to the aseptic removal of a rectangle of ulcer consisting of granulation tissue, border of ulcer and a segment of skin, the area was frozen lightly with ethyl chloride spray (fig. 1 A). In the earlier cases of the series swabs were passed over the ulcer surfaces to secure material for microbial analysis prior to biopsy. In a few other cases the aseptically removed tissue was impressed by contact on suitable mediums for the culture of the organisms present. In the remainder (over half of the cases) the tissue was divided in half; one portion was ground in sterile quartz sand for microbial analysis, and multiple fragments were planted on Sabouraud's medium for mycologic study; the other half of the specimen immediately after removal was fixed in a 4 per cent aqueous solution of formaldehyde, then embedded in paraffin and sectioned serially. Drawings and photographs made during these procedures supplied the necessary data for gross and microscopic correlations in later studies.

Most of our patients were hospitalized. In all instances the patient's residence at the time of the onset of the ulcer was noted, and the elevation above sea level was determined from maps later. The patients tolerated biopsy well. There was no tendency for the operative site to show, other than uneventful healing. In fact, in a few early cases there was rapid involution of the area and of the whole ulcer, healing taking place in several days to a few weeks by formation of a puckered scar. This should not be taken as suggested therapy from our evidence alone, since there were a number of cases in which tropical ulcer in an early stage tended to undergo spontaneous involution on rest in bed alone.

#### CLINICAL ASPECTS

Affecting universally the lower half of the lower leg without predilection for the right as against the left leg, there may be an ulcer varying in size from a fraction of a centimeter to several centimeters in diameter. In the earliest stage seen, in cases of about two weeks' duration, the ulcer tends to be round or oval in outline, with a definitely raised, rolled narrow border of skin enclosing a bright red, finely nodular granulation tissue. The edge is usually slightly undermined, and small deeper pockets of underdipping may be encountered. The edge is epithelized and is directly continuous with unaltered surrounding skin. The surface of the ulcer at this stage is free of exudate; on pressure it may exude a small amount of sanguineous fluid (fig. 1 B). At this stage there is little or no odor. With pro-

gression of the lesion for months, usually accompanied by slow involution, there is a gradual change of color of the granulation tissue surface of the ulcer from bright red to pale pink, along with formation of coarse, irregular nodules. The depressions between nodules may contain small pools of pale yellow to greenish yellow exudate, sometimes putrid. In no instance were adherent or diphtheritic membranes encountered. The edge of the ulcer tends to become flatter with time and assumes a whitish opacity, finally becoming silvery and scarlike. It is at some time during this period that induration, discoloration and flattening of the skin surrounding the edge of the ulcer take place (fig. 1 C). The color changes in the surrounding skin, usually accompanied by mild edema, are pink to red to purple in the earlier stages, becoming brown to bronze with subsequent induration. At the same time the affected skin loses its linear markings, and the hair follicles disappear. Eventually there is formed a flat, evenly depressed, indurated surface (fig. 1 D), which is usually hyperpigmented. Apparently by varying rates of spread and involution, the ulcer assumes an irregular outline. After a duration of many months or years the lesion eventually shows large zones of ulceration lying in the midst of extensive areas of induration and fixation, even to the point of involving the largest part of the lower leg, but rarely exposing underlying muscle or bone. At some time during this phase there appears around the edge of the ulcer an area of depigmentation terminating as an opaque white, irregular zone which gradually extends peripherally (fig. 1 E). At any stage one might see reepithelialization varying in form from buds to bands which tend to grow across the ulcer surface, usually ending abortively. Tenderness, as a rule, is minimal, and in the later stages the skin surrounding the ulcer may be almost anesthetic.

Healing may take place at any of the earlier stages, rarely in the middle stages and probably not at all in the last described. Such healing is frequently spontaneous or occurs in spite of the remedies usually applied. As these areas heal a puckered thin scar is formed. Around the scar is a zone which gradually achieves or has achieved a deep bronze pigmentation, which lasts for years if not indefinitely.

All of the cases studied or surveyed originated in the lowlands (from sea level up to 1,000 feet [300 meters] above sea level), and comprised members of the age groups from the second through the seventh decade. The population studied is predominantly American Indian and Indian-Caucasian. Consequently most of the patients in our series are of that physical type (table 1). In our surveys, however, all types living in the lowlands were found affected: Indian, Caucasian and Negro. No significant difference of incidence was noted between males and females in hospitals or in the field. Occupation appeared to play no predisposing role in the origin of the ulcer.

The medical history was our only approach to an evaluation of the origin of tropical ulcer, with all the vagueness and paucity of detail that the method implies. Fly, mosquito, or other insect bites were indicated as the primary injury at the ulcer site in 6 cases; in 8 cases there was mention of trauma; in 9 cases only an initial "blister," "boil"

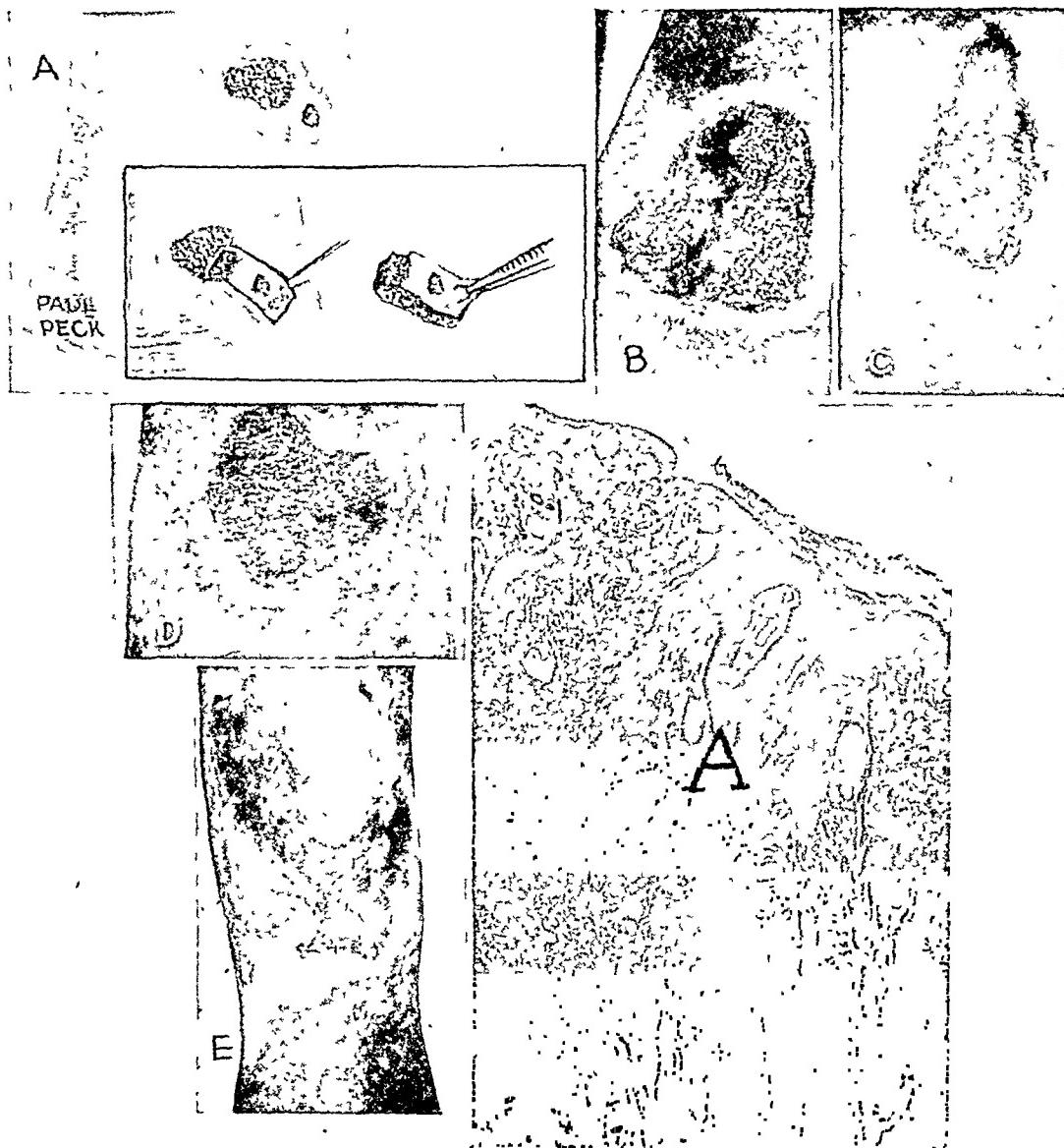


Fig. 1.—*A*, drawing showing the technic of biopsy. The patient (no. 1) presents a satellite ulcer, which was removed with the main biopsy specimen en bloc. *B*, tropical ulcer of fourteen days' duration (patient 17). *C*, tropical ulcer of two years' duration (patient 15). *D*, tropical ulcer of eleven months' duration (patient 16). *E*, tropical ulcer of more than one year's duration (patient 20). *F*, margin of ulcer of patient 17, of fourteen days' duration, showing moderate pseudoepitheliomatous hyperplasia (*A*);  $\times 25$ . (Army Medical Museum negative, no. 89821.)

or "pimple" was reported; in 1 case the remembered details were too vague, or the patient did not know of any antecedent factors. Obviously, no class of injuries was common to these cases at the onset of ulceration.

In all but 1 case the temperature, the pulse rate and the respiratory rate were normal either at the time of the examination or at the time

TABLE 1.—*Data on Patients with Tropical Ulcer*

Patient	Age	Sex	Race	Duration of Lesion	Size of Lesion, Mm.	Bacterial Flora
1	14	F	Indian*	2-3 mo.	10 x 13	No isolations; gram-positive cocci
2	26	F	Negro	1 mo.	34 x 21	No isolations; gram-positive cocci
3	19	F	Negro	1 yr.	22 x 25	Staph. aureus; Microcococcus luteus (?)
4	17	M	Indian	4 mo.	78 x 51	Staph. aureus
5	42	M	Indian	2 mo.	68 x 47	Unidentified gram-positive micrococci; Microcococcus aurantiacus (?)
6	33	M	Indian	3 wk.	9 x 12	Isolations of gram-positive cocci lost before final identification
7	37	M	Indian	3 mo.	13 x 11	Gram-positive cocci (some probably streptococci, others staphylococci—not further identified)
8	20	F	Indian	20 days	13 x 13	Staph. aureus
9	42	F	Indian	1 mo.	10 x 8	Staph. aureus
11	16	F	Indian	2 mo.	25 x 28	Staph. aureus
14	28	M	Indian	5 wk.	51 x 20	Staph. albus; Staph. citreus
15	28	F	Indian	2 yr.	21 x 39	Staph. aureus; Staph. epidermidis; undetermined bacillus (gram-negative nonmotile); a streptococcus of undetermined species
16	18	F	Indian	11 mo.	31 x 33	Streptococcus, species undetermined; Staph. aureus
17	21	M	Indian	14 days	28 x 31	Gram-positive diplococci, not further identified; a gamma hemolytic anaerobic streptococcus, species undetermined
19	17	F	Indian	15 days	16 x 22	Gram-positive bacillus, not further identified; Str. durans (?)
21	17	F	Indian	3 mo.	47 x 56	Staph. aureus; Proteus vulgaris (?)
22	11	F	Indian	2 mo.	7 x 5	Microcococcus luteus (?)
23	25	F	Indian	Undetermined	28 x 18	unidentified gram-positive bacilli
24	17	F	Indian	3 mo.	19 x 24	Staph. aureus; an anaerobic streptococcus of undetermined species
25	14	F	Indian	3 mo.	39 x 9	Proteus vulgaris; Microcococcus luteus (?)
27	56	M	Indian	10 yr.	94 x 34	a diphtheroid
						Bacterium zopfii; a gram-positive bacillus, not further identified; an anaerobic, hemolytic streptococcus of undetermined species
						Diplococcus pneumoniae; gram-negative bacilli; gram-positive bacilli, not further identified
						Staph. albus; Str. haemolyticus; Staph. aureus

\* The term is used to denote all those showing the Central American Indian type of features and general physique. Many of the patients included in this term represented admixtures of Indian and Caucasian types.

the hospital record was made or at both times. Blood pressure readings were taken from the arm and from the legs by the usual auscultatory method. In 6 cases the blood pressures of the legs were at least 10 mm. of mercury higher than those of the arm (i. e., popliteal artery

vs. brachial artery) in the prone and the supine position, respectively. Such differences are within physiologic limits.<sup>1</sup> The highest blood pressure recorded was that of a 17 year old girl (patient 21), being 150 systolic and 65 diastolic. The other patients showed no significant deviations from the normal. No cases of tropical ulcer associated with varicose veins of the legs were included in this series.

No significant abnormalities were noted in the skin of the extremities, particularly in that of the comparable area of the leg not ulcerated, or in the skin of any other part of the body. No lesions were found on physical examination of the thorax of any patient included in this series.

The popliteal lymph nodes were not palpable in any patient studied or in those surveyed, with duration of the lesion ranging from a few weeks to several years. The inguinal lymph nodes were found sometimes to be slightly enlarged but never tender or painful; the nodes on the side of the lesion were no larger than those on the unaffected side. There was no sign of lymphangitis even in the patients with the earliest or most actively progressive ulceration.

The data from the clinical laboratory may be summarized as follows: No abnormalities were encountered in urinalyses. Thick blood smears (usually but one smear per patient) revealed no malarial parasites, although enlargement of the spleen was found in patients 2, 5, 7, 11, 20 and 25, and patient 18 gave a history of splenomegaly with involution. Hemoglobin values varied considerably, as did estimated numbers of red blood cells per cubic millimeter, the usual finding being moderate hypochromic, normocytic anemia. White blood cell counts varied from about 4,000 (leukopenia) to over 18,000 (leukocytosis) per cubic millimeter, with most of the values within normal limits. There was no instance of polymorphonuclear leukocytosis. A fairly constant finding was eosinophilia, the percentage of eosinophils not exceeding 23. In this connection it should be noted that the patients of our series showed a high incidence of intestinal parasitization, even on the basis of single examinations of stools, the most common findings being the ova of *Ascaris lumbricoides*, *Necator americanus* and *Trichuris trichiura*, alone or in combination. No close correlation between the extent of hookworm infection and the degree of anemia was present in this series. The Kahn test disclosed only two doubtful and no positive reactions, but patient 9 had a hospital record of antisyphilitic therapy of one year's duration. Suspensions of the Negro patients' blood cells examined for the sickling trait did not reveal it. Icteric indexes were within normal limits. Sedimentation rates (Wintrobe) revealed no abnormalities when corrected for the anemic state.

1. Yater, W. M.: Fundamentals of Internal Medicine, ed. 2, New York, D. Appleton-Century Company, Inc., 1944, p. 85. Gambill, E. E.; Hines, E. A., Jr.: Am. Heart J. 28:763, 1944.

Analysis of the dietary histories secured from 23 patients revealed no nutritional deficiency common to all of the patients who were studied, but showed considerable individual variation as to qualitative and quantitative food intake. Major William F. Ashe, Medical Corps, Army of the United States, assistant director of the Nutrition Division, Office of the Surgeon General, United States Army, examined our dietary data and rendered the opinion freely quoted. We are convinced that the dietary histories of these patients are representative on the average of those of the general population, i. e., adequate to prevent frank clinical deficiency disease but not optimal.

Neither the patients included in this study nor those surveyed, nor those seen generally in hospitals, showed any clinical signs of vitamin deficiencies, such as blepharitis, xerophthalmia, keratomalacia, vascularization of the cornea, cheilosis, redness or other disfigurement of the tongue, follicular keratoses, erythema of the skin or neuritis.

#### MORBID ANATOMY OF TROPICAL ULCER AS OBSERVED IN GUATEMALA

Serial sections of the specimens were stained by the routine hematoxylin-eosin, Giemsa and Brown-Brenn methods for bacteria in all cases. Step sections were selected for iron staining; the Gallego method as modified by Lillie<sup>2</sup> was used

2. Paraffin sections, after being fixed in neutral 4 per cent aqueous solution of formaldehyde, are passed through the alcohols to water as usual.

Prepare solutions A and B:

A. Hematoxylin, 1 Gm.

95 per cent ethyl alcohol, 100 cc.

B. Solution of ferric chloride U.S.P., 4 cc.

Concentrated hydrochloric acid, 1 cc.

Distilled water, 95 cc.

Mix freshly prepared solutions A and B before use and stain sections six minutes.

Rinse in tap water.

Mordant in:

Distilled water, 200 cc.

Concentrated nitric acid, 1.5 cc.

Solution of formaldehyde U.S.P., 1.0 cc.

Solution of ferric chloride U.S.P., 1.5 cc.

} 30 seconds.

Rinse in tap water.

Stain in:

Carbol fuchsin, 1 per cent solution, 3.0 cc.

Acetic acid, 0.2 per cent aqueous solution, 50 cc.

Mix fresh.

} 5 minutes.

Rinse in distilled water.

Mordant as above—2 minutes.

Stain in trinitrophenol-aniline blue:

Aniline blue, 0.1 Gm.

Saturated aqueous solution of trinitrophenol (picric acid) 100 cc.

} 1 minute.

Rinse in 0.1 per cent aqueous solution of acetic acid.

Dehydrate and clear in acetone, acetone plus xylene, xylene (2 changes).

Mount in balsam.

for connective tissue and for differentiation of elastic tissue; the Masson trichrome stain was used for smooth muscle-connective tissue specific coloration.

For convenience of description the specimens in this series will be grouped as "early" if the duration of ulceration had been about two weeks to two months, "moderately advanced" if the duration had been from a few to several months, and "late" if the duration had been from one to several years.

*Epidermis.*—Epidermal hyperplasia was present in all specimens, being slight and regular in the earlier ones and progressing to fivefold and even tenfold increases of thickness in the late ones. At the margin of the ulcer in the early stages marked pseudoepitheliomatous hyperplasia was seen (fig. 1*F*) but no cancerous proliferation. In the moderately advanced and late stages buds of reepithelialization were noted at the borders, usually thin, and incomplete even in a single section. Occasionally a late specimen showed marked acanthosis at the edge of the ulcer. Hyperparakeratosis was always present at the border, grading to hyperkeratosis more distally, with considerable variation between cases as to degree, and apparently independent of the time element. Flattening of the epidermal surface was another constant feature in this series. Polymorphonuclear leukocyte infiltration of the epidermis, even to the formation of minute intraepidermal abscesses, was an inconstant finding, but as a rule was more pronounced in the early specimens. Decrease of pigment in the basal layer of the epidermis appeared to be a process continuous with the age of the lesion, which finally shows no recognizable pigment cells. (Compare with the clinical description in the foregoing section and with figure 1*E*.)

*Corium.*—The papillae showed elongation, edema, broadening, and blunting of the apices, thus contributing to the flattened contour of the epidermal surface. In the early stages the contents of the papillae consisted mainly of edema fluid, loosened connective tissue, dilated capillaries and hemorrhagic foci. Later there was fibroblastic proliferation increasing to complete fibrosis. There were scattered infiltrating polymorphonuclear leukocytes, with round cell and eosinophilic cell clusters, in the papillae in the early stages; in the later ones the papillae were devoid of leukocytes. Dilatation and tortuosity of capillary loops in the papillae were seen in all sections but were most pronounced in those from the early specimens.

In general, marked exudation of polymorphonuclear leukocytes was seen in the corium in the early stages; exudation predominantly of lymphocytes, monocytes and plasma cells in the moderately advanced ones, and progressively decreasing numbers of those cells in the later stages. The densest leukocyte accumulations were located near the ulcer borders, and graded distally. Foci of recent hemorrhage, siderotic patches, and phagocytes filled with ingested erythrocytes or iron pigment were seen in all of the series with considerable variation between levels of the same specimen. Basophilia was common, scattered at all levels of the corium and the subcutaneous fat and in the granulation tissue of the ulcer bed. Early there was leukocytic disruption of the elements of the pilosebaceous apparatus leading to absolute reduction in numbers in late stages. Foreign body giant cells marked the sites of some destroyed hair shafts, follicles and sebaceous glands. The sweat coils were dilated, had inspissated contents and showed periglandular round cell infiltration and edema. The late specimens showed dense fibrosis of the corium and the subcutaneous fatty layer with disappearance of sweat coils and hair follicles. The nerve bundles in the corium were anatomically normal in some sections; in others they revealed interstitial fibrosis, perineurial round cell infiltration and in moderately advanced stages of the ulcer showed,

in addition, whorling of the nuclei in an almost neuromatous manner. In 2 such cases the nerve segments showed greatly distorted, deeply staining, swollen nuclei; in another, specific staining revealed the myelin disrupted into droplets. The conclusion appears warranted that these nerve changes are the cause of the relative anesthesia noted clinically in the ulcer areas (see section entitled "Clinical Aspects").

*Subcutaneous Fat.*—Serous atrophy, exudation of moderate numbers of polymorphonuclear leukocytes and round cells and, in occasional minute foci, necrosis with foreign body giant cell reactions were seen in the subcutaneous fat.

*Area of Ulcer.*—The ulcerous area underwent minor changes with duration of the lesion. In all stages it consisted of a highly vascularized granulation tissue with the following variations: On the surface was a fibrinopurulent membrane varying from a hardly discernible layer to one the thickness of the epidermis, overlying an irregular zone of hyaline necrosis, sometimes very thin and at others extending for about one quarter of the thickness of the granulation tissue bed. As a rule, the capillaries of the granulation tissue extended to the very surface and early were supported by a very loose fibroblastic matrix; in the later stages fibrosis separated the exuberant vessels into clusters. The inflammatory exudate tended to be predominantly of polymorphonuclear leukocytes in the early specimens and more of the lymphocytic, monocytic and plasma cell type in the later ones. Eosinophil and basophil leukocytes were numerous in early specimens.

*Vascular Involvement.*—The distinguishing feature of all these specimens of tropical ulcer lay in the vascular changes encountered. Arteries, to a lesser extent veins, of all calibers were thickened, their lumens narrowed, sometimes obliterated, occasionally recanalized. The capillaries showed similar changes. Naturally, the vessels of larger caliber were not represented in a few biopsy specimens simply because the block failed to cut across vascular channels of such caliber.

Pathologically, the specimens were different in the three main divisions of the blood supply, namely the arteries, the veins and the capillary bed. The larger, medium-sized and small arteries seen (fig. 2 A and B) showed marked thickening of the media by muscular hyperplasia, with moderate to marked stenosis of the lumen. Such vessels frequently had an intact, well defined internal elastic membrane. In other thickened vessels the internal elastic membrane was split and frayed and had either a band of smooth muscle or a mixture of smooth muscle and collagenous connective tissue central to it, i. e., subintimal fibrosis or simple subintimal hyperplasia. In a few instances the internal elastic membrane was found split and frayed in the middle of a greatly hypertrophied muscular media (fig. 2 C). There was a greatly thickened adventitia as well in many of the smaller arteries. In some cases all of these changes were represented in different vessels at various levels of the same block. In other cases only the commonest of the arterial lesions was seen, namely medial muscular hyperplasia. Serial sections demonstrated the focal nature of the lesion in its extreme form, but rarely did any level demonstrate a restitution of the normal picture.

The larger and medium-sized veins showed inconstantly but frequently either great muscular hyperplasia, with narrowing of the lumen, or a mixture of hyperplasia and interstitial fibrosis. Occasionally a vessel was observed in which the collapse of a wall was outlined by strands of smooth muscle and elastic fibers surrounding a loose collagenous meshwork showing recanalization by capillaries. Or a portion of a venous wall was seen in which occurred a condensation of elastic fibers, an absence of smooth muscle and an admixture of fibroblasts and

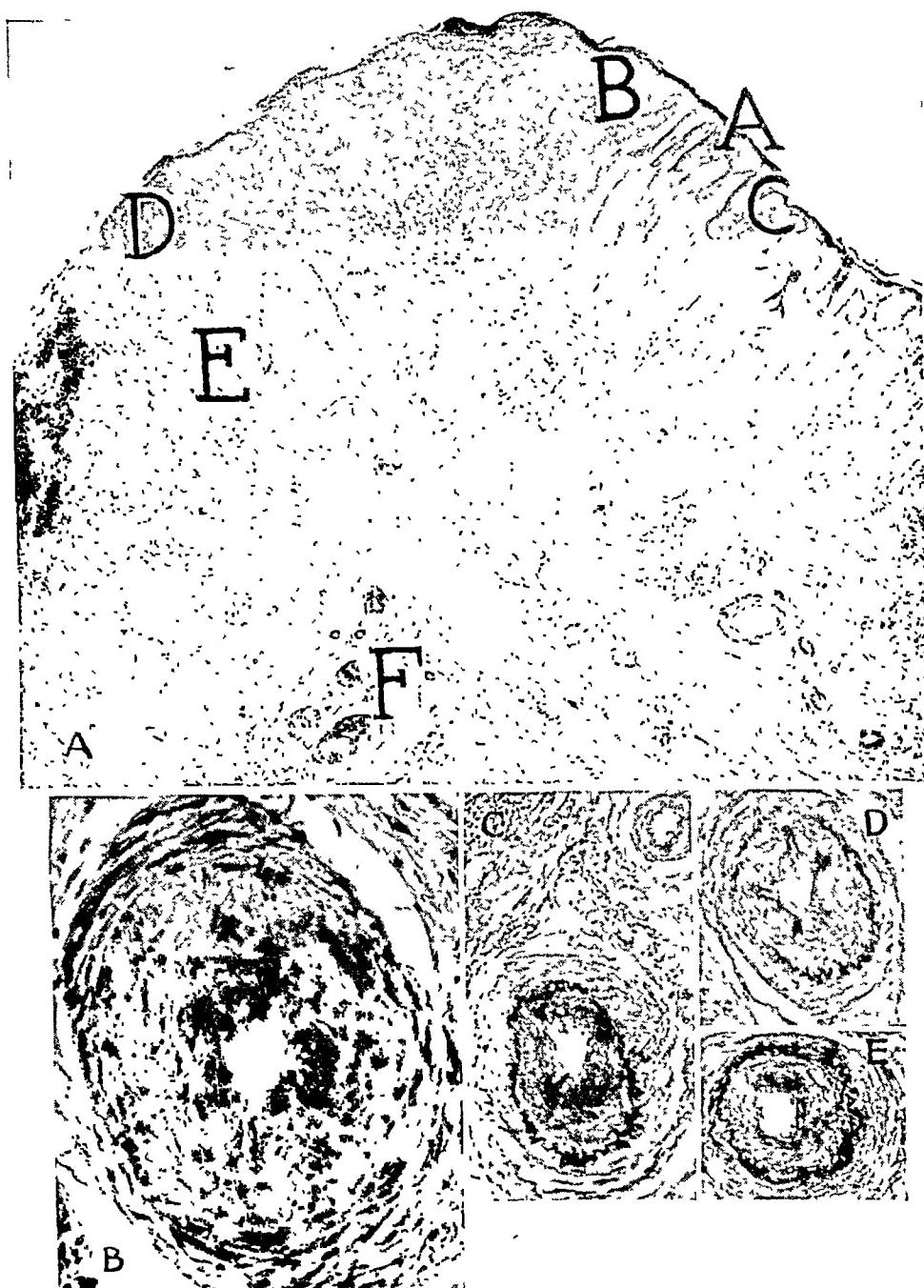


Fig. 2.—*A*, topographic view of a biopsy specimen of tropical ulcer (patient 5) showing the great thickening of the entire skin, the flattened contour of the epidermal surface (*A*), the epidermal hyperplasia (*B*), the broadening and lengthening of the papillae (*C*), the fibrinopurulent membrane over the ulcer surface (*D*), the granulation tissue bed of the ulcer (*E*) and the greatly thickened vessels with narrowed lumens at the level of the subcutaneous adipose tissue (*F*);  $\times 16$ . (Army Medical Museum negative, no. 89820.) *B*, vessel shown at *F* in part *A*;  $\times 230$ . Note the great medial hyperplasia and narrowed lumen. (American Medical Museum negative, no. 89815.) *C*, vessels (*F* in part *A*) stained by the Gallego method as modified by Lillie;  $\times 235$ . The frayed internal elastic laminas now occupy midpositions in the media. (Army Medical Museum negative, no. 89815.)

collagenous fibers. The smaller veins frequently showed not only muscular hyperplasia but adventitial fibrosis.

Not infrequently patchy hyalinization of the wall was seen affecting chiefly, but not exclusively, the smaller arteries, even forming a concentric band (fig. 3). Hyalinization was seen most commonly and fairly uniformly in this series in the capillary bed, where endothelial hyperplasia and hypertrophy were common, resulting in vessels of four and even six endothelial layers. Between such hyperplastic endothelial cells there was often a layer of hyalin (fig. 3). Hyperplasia of endothelium obliterated the lumens of many capillaries.

Vessels of arteriolar caliber showed perhaps the most marked stenosis of any portion of the vascular tree examined, along with muscular hyperplasia, chiefly, and sometimes adventitial fibrosis as well. No tissue preserved in the wet state was available for fat determinations, so it is impossible to state with certainty that the fine vacuolation seen in some such vessel walls represented lipoid infiltration. In one medium-sized artery large lipoidal cells of signet ring form were plentifully distributed through the media.

The following changes were seen once or only a few times in the sections examined: Medium-sized and small arteries showed partial fibrous replacement of the hypertrophied medial coat with and without foci of a gelatinous type of necrosis. Some small veins showed fairly thick bands of subintimal fibrosis narrowing and puckering the lumen. Thrombi, occlusive and nonocclusive, usually fresh, but definitely attached, were seen most frequently in the dilated capillaries of the granulation tissue, but were also seen isolated in one small vein. Lastly, in a few instances special stains demonstrated that clusters of small vessels deep in the corium were in reality a recanalization process in a totally destroyed vessel. A single instance of complete hyalinization of a vessel wall was seen in our oldest specimen, in which a cluster of endothelial cells surrounding a minute lumen was all that remained of the vessel.

The question naturally arises whether these vascular changes are secondary to the inflammatory picture described. The evidence in favor of a primary vascular disease may be summarized as follows:

1. Vascular lesions, including those of greatest severity, were found in all cases of tropical ulcer in an early stage, including those of ten days' to two weeks' duration (fig. 3). In fact, those of years' duration showed great vascular obliteration, apparent only in remnants of smooth muscle and elastic fibers embedded in a much scarred layer of corium or of subcutaneous adipose tissue.

2. In the sections studied the most pronounced vascular changes were found away from the zones of greatest inflammatory exudate, such as the deeper layers of the corium, the subcutaneous adipose tissue and the distal edge of the biopsy block. Very frequently the vessels showing these changes lay in areas entirely devoid of inflammatory exudate, and only rarely were they seen embedded in dense leukocytic pools (fig. 3).

3. One of the two usual vascular diseases secondary to inflammation, whether acute or chronic, suppurative or granulomatous, is endarteritis or endophlebitis, frequently with occlusion. The vascular disease

described in the foregoing paragraphs is entirely different from those entities. The other vascular disease seen secondary to acute or chronic inflammation is vasculitis. The only vessels of this series showing that condition were the much enlarged capillaries of the granulation tissue bed, where acute vasculitis and the deposition of thick, irregular hyaline collars were the rule (fig. 3). These changes are not interpreted as of significance equal to that of the changes described in the foregoing paragraphs, being seen in a variety of inflammatory lesions and in ulcers of varied cause, such as varicose ulcers and those following diabetic arteriosclerosis and trauma.

We have not encountered a similar vascular pattern in any other lesion known to us. That the vascular damage of arteries, veins, arterioles and capillaries should be considered together as a unit seems to us an inescapable conclusion from their association in all cases. It is conceivable that more than one factor operates in the genesis of these vascular abnormalities, a subject which we explored only lightly [see later comment].

By way of making control observations specimens of the skin of the anterior tibial and lateral surfaces of the lower third of the leg were removed from patients coming to routine autopsy in Guatemala who were of the same racial type as most of our patients with ulcer. Males and females, ranging in age from 20 to 50 years, totaling 13, were studied for vascular abnormalities by serial section of specimens of skin of the leg. No vascular changes were found comparable to those of the series with tropical ulcer. (Figure 3 shows typical vessels of the control group.) However, at an autopsy a specimen was secured, consisting of a wide, deeply pigmented zone of the lower extremity, in which there were a few small opaque white plaquelike scars. It is conceivable that this represented a healed tropical ulcer of Guatemala. At any rate, sections of this specimen showed vascular changes comparable in kind but not in degree to those seen in specimens from patient with the ulcer.

One of the other causes of ulceration common in Guatemala is cutaneous leishmaniasis, which does not show any predilection for the lower extremities. Four biopsy specimens from patients with this disease were secured, all representing lesions of many months' duration. They showed no vascular abnormalities at all comparable to those of the tropical ulcer series. Furthermore, they showed a pronounced granulomatous inflammation differing again from that seen in the tropical ulcer series. Leishmania forms were identified after prolonged search in one section from each of these serially sectioned specimens.

Another biopsy specimen was secured in Guatemala from a varicose ulcer of a lower extremity of a man who had had varicosities for twelve years and the ulcer for about one year. The Trendelenburg test showed reflux filling of the distended veins from above downward. The varicosi-

PATIENT AGE SEX DURATION LOCATION  
NO. OF LESION OF VESSELS SHOWN

1 14 F 2-3 MO. 3



H.P.

19 17 F 15D. 4



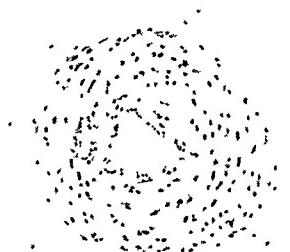
H.P.

17 21 M 14D. 3



H.P.

2 26 F 1MO. 4



L.P.

4 17 M 4MO. 2 & 3



H.P.

H.P.

6 23 M 22D. 1



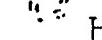
H.P.

3 19 F 1YR. 3



H.P.

NORMAL 2



H.P.

H.P.



LOW POWER 1 2 .3 4 5 6 7 8 9 1 mm.

HIGH POWER 1 .1 .2 mm.

Figure 3

(See legend on opposite page)

ties were plainly visible and markedly severe. There were gross differences between this specimen of ulcer and those of our tropical ulcer series. The microscopic sections of the varicose ulcer showed none of the vascular changes described by us as a distinguishing feature of tropical ulcer.

From the files of the pathology laboratory of the National Institute of Health, Bethesda, Md., were selected 5 cases<sup>3</sup> of ulceration of the lower leg originating in the continental United States in patients ranging from 13 to 59 years of age, none of which showed comparable vascular lesions. In a sixth case,<sup>4</sup> ulceration of the lower leg from the same source occurred in a steamship wireless operator who had spent ten months in the South Pacific Area. The vessels seen in one of the biopsies were only of small artery and arteriolar caliber but were comparable to those seen in our tropical ulcer series in their thickenings and luminal stenoses.

Inasmuch as other investigators (see the following section) have reported fusospirillosis of the type observed in Vincent's infection as the causative agent of tropical ulcer, one of us (A. G.) examined a series of 46 biopsy specimens secured by Dr. E. Hampp,<sup>5</sup> passed assis-

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3. S-21140, S-21057, S-20478, S-19991, S-19812.

4. S-19642, and S-19396. All of these cases were studied with the permission of Dr. R. D. Lillie.

5. Unpublished data.

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#### EXPLANATION OF FIGURE 3

Camera lucida drawings of affected blood vessels. The patients are those of the same numbers listed in tables 1 and 2. The location of the vessels illustrated is given in numerical zones, which are diagrammatically represented in the figure at the lower left hand corner. *H. P.* signifies high power ( $\times 440$ ) and *L. P.* low power. ( $\times 100$ ).

Patient 1. The medium-sized and large vessels (arteries) show muscular hyperplasia of the media and marked stenosis of the lumen. The smallest vessel is a capillary showing endothelial hyperplasia and hyaline thickening of the wall.

Patients 17 and 19. A small artery and an arteriole are illustrated, respectively, both showing muscular hyperplasia of the media with encroachment on the lumen.

Patient 2. This is a medium-sized vein showing muscular hyperplasia of the wall and interstitial fibrosis of the media, resulting in an irregular, narrowed lumen.

Patient 4. The two smaller vessels are capillaries showing extreme hyaline thickening of the walls and marked endothelial proliferation. The largest vessel is a small artery with lesions like those already described.

Patient 6. This is a capillary in the granulation tissue with a thick, irregular hyaline collar around it, permeated by polymorphonuclear leukocytes.

Patient 3. The stenotic lumen of this vessel is surrounded by a wide band of hyaline necrosis within the muscle coat.

"Normal" represents a group of three vessels as seen in one of the control sections of skin removed at autopsy from the lower third of the leg.

tant surgeon, United States Public Health Service, in known cases of Vincent's gingivitis. None of these sections showed any vascular abnormalities at all comparable to those of our tropical ulcer series.

*Bony Involvement.*—Another feature of tropical ulcer occurring in this region is the eventual involvement of bone that takes place in the chronic cases, i. e., those of many months' to years' duration. Two patients with involvement of bone are included in this series, patients 20 and 27. In the former a roentgenogram taken prior to operative exploration showed marked cortical opacity and thickening which encroached on the medullary canal, periosteal thickening and fine opaque radial lines, which were interpreted as calcifications. Microscopic sections of the bone showed greatly thickened lamellas, without evidence of active osteoclasia or osteoplasia. The marrow elements were reduced to a loose collagenous meshwork, in which very small numbers of round cells were identified. There was no evidence of osteomyelitis.

*Negative Findings.*—There are a number of significant negative findings that require emphasis; no granulomas other than the small numbers of foreign body giant cells described in a foregoing paragraph (page 619) were encountered; no fungous forms were found; no inclusion bodies were seen; no parasites were found. The female chigoe (*Tunga penetrans*) is a common parasite in Guatemala infecting as a rule the toes, occasionally the hands, in a characteristic paronychial distribution. It so happened that none of the patients in this series was infected by chigoës, and none was seen grossly or microscopically in the specimens of tropical ulcer. The vascular changes described and illustrated served to differentiate this from the other ulcers of the area studied.

#### MICRO-ORGANISMAL FLORA OF GUATEMALAN TROPICAL ULCER

According to the general plan of procedure already outlined, cultures were made on blood agar aerobically and anaerobically (Wright's method). Increased carbon dioxide tension was used for blood agar slants in several cases but showed no advantages and its use as a routine procedure was later discontinued. Robertson's cooked meat medium (with 0.5 per cent tryptose added; sealed with a petrolatum plug), brain broth medium (Bacto), semisolid thioglycollate stab preparations (Brewer's) and potassium tellurite-blood agar plates were also used.

The results are summarized in table 1. Different varieties of staphylococci and streptococci were identified, with no constancy as to species, along with a number of coccal and bacillary forms known to be nonpathogenic. No significance could be attached to differences in flora in those several instances in which excised corium and ulcer surface were cultured separately. The varying character of the bacterial flora was brought out clearly in a series of 26 cases in which smear preparations were made directly from the ulcer, in 20 of which special mediums were used for the isolation of organisms of the Corynebacteria group (see table 2 for a report of the latter). In contrast to the reports of many investigators in other parts of the world, notably Clements,<sup>6</sup> who found Vincent's

6. Clements, W.: M. J. Australia 2:615, 1936.

fusiform bacillus-spirochetal complex regularly in tropical ulcer, we found it not at all as a complete picture. Occasionally spirilla and occasional bacillary forms were found admixed among coccal and fine bacillary forms. The varicose ulcer described on page 623 and a bacterial flora similar to that of the tropical ulcer series, consisting, namely, of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Cultures for fungi were made from triturated portions of the biopsy specimens. In a few cases cultures were made by mincing a part of the specimen with sterile scissors, and in the remainder cultures were made also from the surface of the ulcer.

The inoculum was streaked on the surface of Sabouraud's agar slants. Deep shake cultures on dextrose-veal infusion-agar also were made in a few of the cases. Cultures were incubated two months before being discarded.

In most of the cases a yellow staphylococcus was isolated. There were no consistent mycologic findings, and it was concluded that the few miscellaneous molds cultured were accidental contaminants. The results of the attempts to isolate fungi are given in table 2.

TABLE 2.—*Results of Attempts to Isolate Fungi from Areas of Tropical Ulcer in Twenty-Four Patients*

Patient	Fungi	Patient.....	Fungi	Patient	Fungi
1.....	0	9.....	0	19	<i>Candida tropicalis</i>
2.....	0	10.....	0	21	0
3.....	0	11.....	0	22	0
4.....	0	14.....	0	23	<i>Cladosporium SP.</i>
5.....	0	15.....	<i>Monilia SP.</i>	24	0
6.....	0	16.....	0	25	<i>Neurospora sitophila</i>
7.....	0	17.....	<i>Streptomyces</i>	27	Sterile gray mold
8.....	0	18.....	0	29	0 (Varicose ulcer)

The tissue sections were examined for micro-organisms by the Brown-Brenn and the Giemsa method, with morphologic findings consistent with those listed in table 1. Most of the organisms seen in tissue sections were on the surface in sparse numbers, occasionally in minute clumps. Small numbers of exclusively gram-positive cocci could be seen deep in the granulation tissue bed of the ulcer area, and occasionally phagocytosis. Considerable difficulty was experienced in differentiating some basophils and cells with granular debris in their cytoplasm from phagocytes with ingested bacteria. Usually tissue basophils with small uniform granules had a purplish hue with both the Giemsa and the Brown-Brenn method, while the gram-positive bacteria were definitely blue with both methods. Ten sections from 10 patients were stained by the Warthin-Starry method for spirochetes in an attempt to find organisms of Vincent's complex. None were seen.

Even though the organisms isolated from these patients were of the usual skin-contaminant group it was deemed essential to test their pathogenicity. Inoculations of pure cultures of isolated staphylococci and streptococci and of both organisms together were made intracutaneously in guinea pigs and rabbits. All failed to produce any lesion other than a temporary inflammatory zone without ulceration or any entity at all comparable to the tropical ulcer studied in man. Furthermore, in all the animals the lesions healed in several days to a few weeks at the most, thus differing further, in lack of chronicity, from the human lesions; 35 guinea pigs, 16 rabbits and 1 monkey were used in these experiments.

Proceeding on the possibility that a noncultivable agent, such as a filtrable virus, might be present, 1 monkey (*Macacus rhesus*), 4 rabbits and 4 guinea pigs were inoculated with fresh suspensions of 4 pooled triturated biopsy specimens of tropical ulcer without producing any lesions whatsoever in thirty days of observation.

It was decided to make a special search for organisms of the *Corynebacteria* group, using a series of patients with tropical ulcer in one hospital, not previously studied. Of 20 patients surveyed, 1 was found harboring diphtheria organisms pathogenic for the guinea pig, confirmed by autopsy observation of typical myocardial lesions and extreme adrenal hemorrhage. This patient's ulcer differed not at all clinically or grossly from those previously studied. Nor did this patient have any clinical signs of diphtheria intoxication. One hundred thousand units of diphtheria antitoxin was administered intramuscularly in a single dose to this patient after the character of the isolated micro-organisms had been established. There was no clinical improvement in the ulcer thereafter over a two week period of observation. Time did not permit us to reassay the lesion for diphtheria organisms after administration of the specific antitoxin.

It appeared entirely consistent with the observed facts to conclude that the micro-organisms isolated were harbored by the lesions but were not etiologically related. The lack of constant bacterial types or even of common combinations of organisms in all lesions supported this conclusion. The demonstrated lack of pathogenicity of the organisms inoculated in experimental animals was further support for this thesis.

Proteus agglutinations were undertaken with stock cultures of O X 19, O X 2, O X L and O X K, which revealed no significant titers for any of the serums, although cases of months' and even years' duration were included in this series. This does not necessarily exclude an organism of the *Rickettsia* group from etiologic consideration, since species of the Q fever type, for example, do not react with the Proteus antigens.

#### COMMENT

Of the experimental evidence presented no one type stands out as clearly as the vascular, seen in all cases on microscopic examination of the tissue sections. It is the factor common to all. Furthermore, the vascular damage is sufficiently severe to warrant the conclusion that there is serious interference with the blood supply of the region. Such a circulatory deficiency is known to result in ulceration whether it be on the basis of arteriosclerosis or that of varicose veins.

We were unsuccessful in our search for factors productive of the vascular changes. Hypertension could be dismissed as an etiologic agent by reason of our measurements which, even though they were single readings, covered a much larger group than that represented in this series. Because our patients so frequently gave a history of "fly bites," we attempted to produce experimental insect sting trauma. Guinea pigs and rabbits were subjected to the bites of the tick *Boophilus emulatus*; others were observed while laboratory bred *Triatoma* fed fully on their shaved skin; another was stung by a honey bee. In all not even an appreciable wheal was produced. Before surrendering this bit of speculation, we wish to point out that insect bites sustained

by one of us (A. G.) in the field were frequently much more severe on the lower legs than anywhere else on the body. As a general observation, the population of the lowlands as seen in market places, at work and in dispensaries tended to show more severe reactions to insect bites on the lower extremities than elsewhere on the body. Whether the intensity of these reactions is correlated with some vascular peculiarity of the region remains to be proved. Earle<sup>7</sup> quoted Rauber and Kopsch to the effect that the integument of the lower third of the leg is supplied by terminal vessels of adjoining arteries. Whatever may be the underlying anatomic basis, if any exists, the fact remains that the bodily region so commonly affected by tropical ulcer is vulnerable to the consequences of vascular insufficiencies whether these are produced by low temperatures, occlusive arteriosclerosis or failure of adequate venous return.

March and Wilson<sup>8</sup> summed up their experience with tropical ulcer as observed in Iran with the "alliterative mnemonic" as to etiology: "Filth, food, friction, and fusospirillosis." We were not impressed with lack of cleanliness in our patients. In fact, many showed reasonable maintenance of hygienic conditions. "Food," meaning various nutritional deficiencies, has been discussed at length by many authors, among whom Earle,<sup>7</sup> studying tropical ulcer in Trinidad, postulates deficiencies of dietary animal protein, animal fat, calcium, phosphorus and vitamins A, B<sub>1</sub>, B<sub>2</sub>, C and D as contributory factors in the genesis of the lesion. Clements<sup>6</sup> incriminated as one of the causal agents another dietary complex as seen in New Guinea: partial or complete deficiency of the B<sub>2</sub> complex, abundance of carbohydrates and only small amounts of proteins and fats. Charters<sup>9</sup> expressed the belief that vitamin A deficiency is a precursor of the "specific infection" of tropical ulcer. Whatever validity those dietary observations have they fail to agree as to specificity. In addition, our dietary findings are not in agreement with them. Our patients ate what can be compared biochemically to a diet encountered among the poor of the Southern states. It appears reasonable to inquire why, if dietary deficiency is the cause of tropical ulcer, it is not seen in that area. Furthermore, if vitamin A deficiency, for example, is a significant contributing factor, why is tropical ulcer not seen in many other parts of the world where that deficiency is certainly not unknown? If all that is needed is superimposed bacterial infection, the micro-organismal flora seen by us and that seen by others as fusospirillosis is ubiquitous.

7. Earle, K. V.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **35**:241, 1942.

8. March, F., and Wilson, H. A.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **38**: 259, 1945.

9. Charters, A. D.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **37**:205, 1943.

Our method of clinical and laboratory examination of the patient, survey of the micro-organisms and microscopic study of the tissues secured by biopsy has yielded significant information as to the character of tropical ulcer as observed in one geographic area. We have no experience with it as seen in other parts of the world. Consequently we cannot generalize on our findings or criticize the findings of those working in other countries.

The therapy of tropical ulcer has been directed toward bacterial stasis or antisepsis, without great success. It would appear from our observations that methods aimed at restoring the adequacy of the blood supply of the leg would prove more fruitful therapeutically.

#### SUMMARY

The tropical ulcer of Guatemala originates in the lowlands. In this study designed to secure data on its genesis, clinical methods and clinical laboratory methods revealed nothing of fundamental importance. Bacteriologic and mycologic technics demonstrated a micro-organismal flora of nonuniform type, regarded as adventitious. Fresh ulcer tissue and pure growths of the isolated bacteria when inoculated in rabbits, guinea pigs and a monkey produced no lesions at all comparable to the tropical ulcer of the area. The significant lesion in these cases of tropical ulcer was found on microscopic examination of biopsy specimens and consisted of widespread damage of the venous and arterial supply. While the vascular abnormalities were of variable anatomic type they had one characteristic in common: stenosis of the lumen. An argument is presented for regarding these vascular abnormalities as the primary cause of this ulceration.

## ETHYLENE GLYCOL POISONING

With Suggestions for Its Treatment as Oxalate Poisoning

COMMANDER GEORGE MILLES (MC), U.S.N.R.

THE GLYCOLS are stock commercial solvents, and ethylene glycol is freely accessible to the general population in the form of an anti-freeze solution. The toxicity of this group of compounds was forced on the public consciousness by the fatalities which occurred incident to the use of a solution of sulfanilamide in diethylene glycol. This compound, in common with others of the group having an ether linkage, causes hydropic degeneration of the renal tubular epithelium.<sup>1</sup>

The toxicity of ethylene glycol has not received the publicity that it merits. Indeed, the containers in which this glycol is marketed bear no indication of the fact that it is poisonous. In 1927 Page<sup>2</sup> published a pharmacologic study of this compound which indicated that it was relatively nontoxic. He drank 15 cc. without ill effect. He reported that dogs tolerated a 30 per cent solution in a dose of 9 cc. per kilogram of body weight without apparent effect and that the concentrated product in a dose of 5 cc. per kilogram of body weight caused only emesis. He stated that because of the structure of ethylene glycol it would not seem unlikely that the compound would be converted to sugar in the animal body.

In 1930, in a letter<sup>3</sup> reporting the death of 2 men following their ingestion of ethylene glycol (Prestone) it was stated: "Death seemed to have been caused by respiratory failure and convulsions." The victims were first seen twelve hours after they had drunk diluted antifreeze solution. They were stuporous; their respirations were rapid; the skin was cold and somewhat cyanotic. Vomiting was persistent, and prostration was extreme. The anatomic findings were not reported.

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This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

1. Kesten, H. D.; Mulinos, M. G., and Pomerantz, L.: J. A. M. A. **109**: 1509, 1937.

2. Page, I. H.: J. Pharmacol. & Exper. Therap. **30**:313, 1927.

3. Possible Death from Drinking Ethylene Glycol ("Prestone"), Queries and Minor Notes, J. A. M. A. **94**:1940, 1930.

Brekke<sup>4</sup> reported 2 cases of ethylene glycol poisoning, with recovery following decapsulation of the kidneys. The initial symptoms were similar to those of alcoholic intoxication. They were followed by paralysis of some cerebral nerves, hemorrhagic nephritis, acute uremia and marked hyperemia and swelling of the kidneys. Wiley and associates<sup>5</sup> found that dogs and rabbits oxidized glyoxal, glycolic acid, ethylene glycol and ethylene glycol monacetate to oxalic acid.

Laug and associates<sup>6</sup> stated that ethylene glycol caused congestion and hemorrhage of the lungs, albuminuria and hydropic degeneration of the tubular epithelium of the kidneys.

Sollmann<sup>7</sup> described ethylene glycol as being twice as toxic as propylene glycol and half as toxic as diethylene glycol. He stated that when ethylene glycol is oxidized to oxalic acid, but a small fraction of that consumed appears to be involved and that oxidation cannot account for its toxicity.

Lehmann and Flury<sup>8</sup> stated that many authors have ascribed the effect of ethylene glycol to the formation of oxalic acid and that 3 per cent of the ingested ethylene glycol can be detected in the urine in the form of oxalic acid. They concluded that because it is oxidized to oxalic acid, ethylene glycol is more toxic than propylene glycol, since the latter is oxidized to pyruvic acid or lactic acid, and is eliminated in combination with glycuronic acid. They described the effect of large doses of ethylene glycol as exciting and later depressing the central nervous system, and the lethal dose as ranging from 5 to 9 cc. per kilogram of body weight of mice, rats and rabbits, the commercial being more toxic than an especially highly purified product.

Boemke<sup>9</sup> reported an instance in which a bucketful of antifreeze solution was substituted for the coffee water in a German military unit. It was noted that the coffee had a sweetish, somewhat bitter taste. During the course of the morning various members of the unit complained of headache and became intoxicated, for which they thought the coffee responsible. Two of the men who drank especially large amounts of the coffee were admitted to the hospital, severely intoxicated, dizzy and dyspneic. Their alae nasi moved actively, their facial expression

4. Brekke, A.: Norsk mag. f. lægevidensk. **91**:381, 1930.

5. Wiley, F. H.; Hueper, W. C., and von Oettingen, W. F.: J. Indust. Hyg. & Toxicol. **18**:123, 1936.

6. Laug, E. P.; Calver, H. O.; Morris, H. J., and Woodward, G.: J. Indust. Hyg. & Toxicol. **21**:173, 1939.

7. Sollmann, T.: A Manual of Pharmacology, ed. 6, Philadelphia, W. B. Saunders Company, 1942, p. 941.

8. Lehmann, K. B., and Flury, F.: Toxicology and Hygiene of Industrial Solvents, Baltimore, Williams & Wilkins Company, 1943, p. 254.

9. Boemke, F.: Virchows Arch. f. path. Anat. **310**:106, 1943.

was one of fright and their reflexes were depressed. One, who was 30 years old, rapidly lapsed into unconsciousness, became anuric and died on the day following his admission. At autopsy the findings were not distinctive. Microscopically, the kidneys alone were noteworthy. The tubules were dilated, and the tubular epithelium contained finely divided fat droplets. The lumens contained hyaline material, erythrocytes and crystals of various types, stained faintly blue with hematoxylin. The second patient, who was 24 years old, improved somewhat under symptomatic treatment. He passed small amounts of urine containing albumin and red blood cells but no oxalate crystals. He became anuric, lapsed into unconsciousness and died ten days after ingesting the contaminated coffee. At autopsy the findings were hemorrhagic bronchitis and bronchopneumonia, fibrinous pleurisy of the right lower pulmonary lobe, dilatation of the right side of the heart and edema of the brain. The kidneys were swollen and pale. The microscopic changes in the kidneys were similar to those described in the first case. Since these men were truck drivers, carbon monoxide poisoning was suspected at the time of their admission, and spectroscopic examination of the blood disclosed the presence of carbon monoxide hemoglobin. However, the coffee was shown to contain 10 per cent ethylene glycol, and in view of the presence of oxalate crystals in the kidneys, this was recognized as the cause of death. The author referred to two other deaths which occurred one day after the ingestion of 750 cc. of ethylene glycol in which the anatomic observations were the same as in the cases just described. He ascribed deaths from the ingestion of ethylene glycol to the fact that this compound is oxidized to oxalic acid, which is deposited in the kidneys, causing anuria and uremia.

My interest in the toxicity of ethylene glycol was aroused by the following case:

#### REPORT OF A CASE

A 30 year old white man was admitted to the hospital with a history of having drunk antifreeze solution about four hours previously. During the interval prior to admission he became euphoric and vomited. On admission his voice was husky, his speech was thick and he was confused. The conjunctivas and the pharynx were injected, and the skin was cyanotic. The blood pressure was 128 systolic and 90 diastolic; the pulse was full and regular and the rate 90; the pupils were sluggish. About seven hours after he ingested the radiator fluid, his respirations were labored, and the rate was 36 per minute; the pulse had increased to 120, and he vomited foamy yellow fluid. Evidence of pulmonary edema became increasingly pronounced, and the patient lapsed into coma ten hours after drinking the antifreeze solution. At this time, the blood pressure was 172 systolic and 100 diastolic, respirations were sighing and rapid, and the fingers and the hands had become more deeply cyanotic, though the heart tones were good. Frothy, blood-stained fluid was aspirated from the air passages. Twenty-one hours after the ingestion of the radiator fluid the axillary temperature had risen to 101.4 F. The patient had voided 1,650 cc. of urine since his admission. It contained 20 mg.

of albumin and numerous oxalate and urate crystals. He died about twenty-two hours after ingesting the fluid.

Traces of ethylene glycol were demonstrated in the stomach contents aspirated about ten hours after its ingestion. A sample of the radiator fluid submitted for analysis was emerald green and was found to contain about 50 per cent ethylene glycol. No other common poison could be demonstrated. Inquiry indicated that the man had drunk not more than 500 cc. of the fluid.

Treatment was symptomatic and included intravenous injection of sodium *r*-lactate one-sixth molar N. N. R., to combat the apparent acidosis, and gastric lavage.

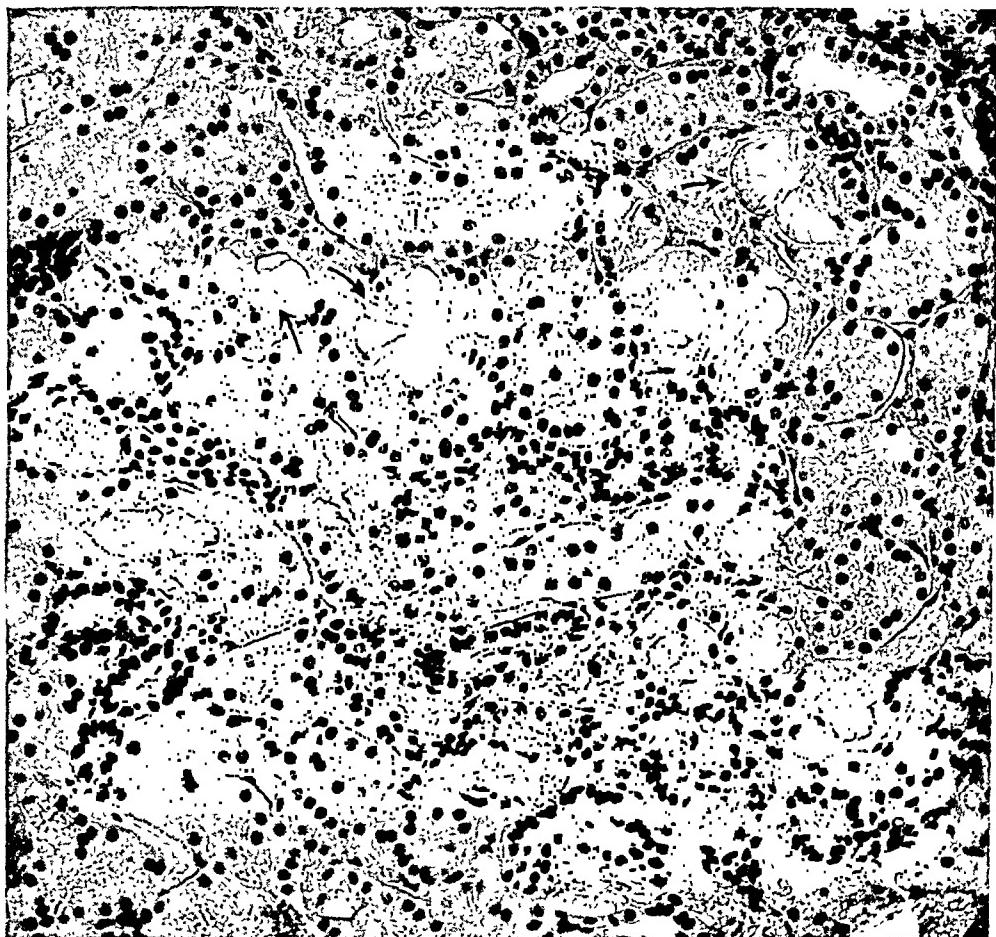


Fig. 1.—Oxalate crystals in the renal tubules of a 30 year old man dead from ingestion of ethylene glycol.

At autopsy, eight hours after death, the right pupil was slightly larger than the left, the conjunctivas were injected and edematous, and the skin and the mucous membranes of the head and the neck were cyanotic. The lividity of dependent parts was unusually pronounced. The heart weighed 410 Gm. and was distended with postmortem clots. The distal end of the trachea and both bronchial trees were filled with blood-stained serous fluid, and their mucosa was red. The lungs were boggy; their dark red cut surfaces pitted on pressure and exuded an abundance of blood-stained serous fluid. The lining surface of the

esophagus and stomach was homogeneously pale, opaque and grayish tan. There were a few mucosal hemorrhages in the stomach. Several blanched peanuts found in the esophagus and the stomach were stained the same shade of green as the fluid which was said to have been ingested. The stomach and small and large intestines were distended with light, muddy, greenish brown, thick fluid. The liver was light reddish brown mottled with yellow and was normal in size and consistency. The kidneys together weighed 350 Gm. They appeared grossly normal except for their rather pale color. The vessels of the pia-arachnoid were markedly congested. There was pronounced lipping of several thoracic vertebrae. The autopsy findings were otherwise not remarkable. Postmortem determinations of blood sugar and nonprotein nitrogen showed 34 mg. and 74 mg. per hundred cubic centimeters respectively.

On microscopic examination of the kidney, the glomerular tufts were seen to be moderately distended and their capillaries filled with red cells. Their interstitial tissue had a coarsely fibrillar, hyaline appearance; its nuclei were deeply stained and angular. Bowman's capsule was somewhat increased in thickness and hyalinized. The epithelium of the convoluted tubules was swollen except at the many points where the tubules were distended with colorless rosette and linear crystals. Similar crystals were present in the collecting tubules (fig. 1). The liver cells were swollen and honeycombed with fine fat vacuoles, which became confluent in the cells about the central veins. Their nuclei were variable. The Kupffer cells contained an abundance of brown granular pigment. The pulmonary alveoli were filled with serous material, and in focal areas they and their neighboring bronchioles were filled with an exudate of polymorphonuclear leukocytes. Congestion and, to a lesser extent, edema characterized the pathologic process in the other organs. Death was due to edema of the lungs, which may have been a condition secondary to an aggravating factor in or the cause of the marked myocardial dilatation and evident acute myocardial failure.

#### EXPERIMENTAL STUDY

The fluid which this man was said to have drunk was administered to several cats by stomach tube.

*Cat 1.*—A male cat weighing 3.25 Kg. was given a dose of 1 cc. per kilogram. Its condition as observed at intervals after administration of the fluid was as follows:

20 min.	Drowsy
30 min.	Less drowsy but apathetic
36 min.	Responsive to the smell of food (sat up)
46 min.	Apathetic, hiccupped
3 hr.	Dozing, apathetic
23 hr.	Apathetic, dozing, responsive to pain and noise, slightly unsteady of gait
27 hr.	Alert, tense, with good muscle coordination, somewhat apathetic
48 hr.	Normal

*Cat 2.*—A female cat weighing 2.4 Kg. was given a dose of 3 cc. per kilogram. Its condition as noted at intervals after administration of the fluid was as follows:

3 hr.	Slightly depressed
22	Practically normal
29	Irritable
46	Irritable, tender over abdomen and flanks
94	Slightly depressed, with no tenderness
118	Apparently normal
142	Comatose
144	Seized with clonic and tonic convulsions
146	Dead, with colorless froth at mouth

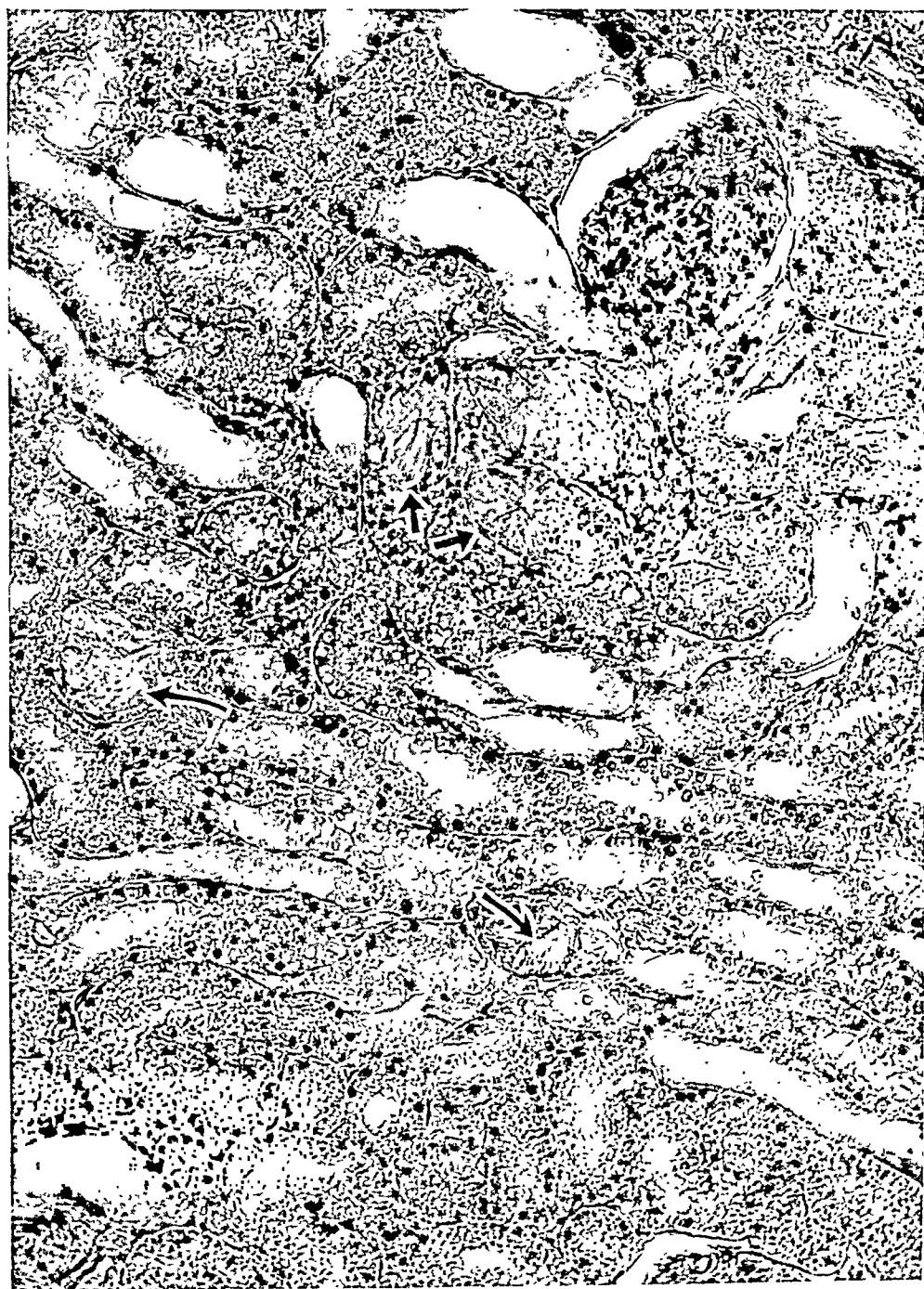


Fig. 2.—Oxalate crystals in the renal tubules of a cat dead one hundred and forty-six hours after oral administration of 3 cc. of 50 per cent ethylene glycol per kilogram of body weight.

Autopsy showed hyperemia and edema of the lungs, concentric dilatation of the heart and cloudy swelling of the kidneys.

Microscopic examination revealed numerous linear crystals in the renal tubules; the tubular epithelium was flat, and its cytoplasm was scanty and hyalinized (fig. 2).

*Cat 3.*—A male cat weighing 3.3 Kg. was given a dose of 5 cc. per kilogram. The protocol of this animal follows.

Immediately	Salivating
10 min.	Dozing, apathetic, salivating
20 min.	Dozing (salivation stopped)
40 min.	Responsive to smell of food (stands up alert)
45 min.	Dozing, apathetic
23 hr.	Comatose, not responsive to pain or noise, with shallow, rapid respirations and widely dilated pupils
26 hr.	Comatose, with shallow, rapid respirations, dilated pupils reacting weakly to light, brisk reflexes, marked analgesia; cat goes into extensor spasm on being handled
29 hr.	Convulsive; showing spontaneous tonic and clonic convulsions; completely analgesic
31 hr.	Briefly convulsive at infrequent intervals
36 hr.	Deeply comatose; cat died; no autopsy

*Cat 4.*—A female cat weighing 1.9 Kg. was given a dose of 5 cc. per kilogram. The condition of the animal at intervals after administration of the fluid was as follows:

1 hr.	Apathetic, depressed
4	Markedly ataxic, moderately depressed, slightly analgesic
23	Apathetic, ataxic, analgesic
27	Lying on side, conscious; when stimulated it stands for a moment, then falls; sits only briefly; analgesic
48	Paralyzed, conscious, with fair tendon and corneal reflexes
52	Killed by blow

The observations at autopsy on microscopic examination were essentially the same as those recorded for cat 2.

#### COMMENT

Ethylene glycol when ingested causes edema of the lungs, dilatation of the heart and a tendency toward bronchopneumonia but leaves its most significant imprint on the kidneys in the form of abundant deposits of oxylate crystals and evidence of degeneration of the epithelium of the convoluted tubules. Clinically its effect is characterized by (*a*) transient stimulation followed by depression of the central nervous system, (*b*) evidence of renal damage, which may proceed to anuria and uremia, (*c*) acute cardiac failure and (*d*) pulmonary edema.

The heavy deposits of crystals in the kidneys suggest that the amount of ethylene glycol which is oxidized to oxalic acid determines its toxicity. It is evident that this opinion and these observations are in conflict with those of some other authors. The discrepancy may be due to their lack of microscopic studies, or to the possibility that oxidation of ethylene glycol is centered in the liver. The latter hypothesis is based on the fact that, taken orally, all of the ingested glycol passes through the liver, whereas when the compound is given intravenously or subcutaneously, the liver is by-passed by a large part of it.

The important role that oxalic acid may play in the toxic effects of ethylene glycol suggests that treatment should be directed toward the relief of oxalate poisoning. Calcium has been recognized as the most effective antidote for oxalate poisoning; an extract of parathyroid gland was proved effective by Kochmann;<sup>10</sup> insulin was found to increase the rate of oxidation of oxalic acid in the body by De Lucia.<sup>11</sup> The desperate state of the person who has ingested any considerable amount of ethylene glycol justifies the use of all of these measures. The tendency toward acute dilatation of the heart and pulmonary edema suggests that great care be used in administering fluid intravenously in any considerable amount or at any but an extremely slow rate.

Experimental studies to determine the effectiveness of the method of treatment indicated should be undertaken with adequate controls and determinations of the blood oxalate level, the urinary oxalate output, the blood calcium and sugar, the blood and urinary  $p_H$  and the effect of large volumes of fluid given intravenously on the incidence of pulmonary edema and cardiac failure in animals poisoned with ethylene glycol.

#### SUMMARY

In man ethylene glycol is a rapidly acting poison; the fatal dose for cats is as low as 1.5 cc. per kilogram (3 cc. per kilogram of anti-freeze fluid containing ethylene glycol in the concentration of 50 per cent).

Anatomic evidence suggests that ethylene glycol taken by mouth is rapidly oxidized to oxalic acid. Oxalate crystals are deposited in abundance in the renal tubules.

Death may result from renal failure or acute cardiac failure and pulmonary edema.

Treatment directed at oxalate poisoning is suggested.

Ethylene glycol is poisonous when ingested and the container in which it is sold should be so labeled.

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10. Kochmann, M.: Deutsche med. Wchnschr. **60**:406, 1934.

11. De Lucia, P.: Boll. Soc. ital. biol. sper. **4**:756, 1929.

## ETIOLOGIC CONCEPTS AND PATHOLOGIC ASPECTS OF AINHUM

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SINCE 1860, when Clark<sup>1</sup> reported on "dry gangrene" occurring in the small toes of Negroes, the condition, later formally described and called ainhum by da Silva Lima,<sup>2</sup> has intrigued clinicians and pathologists. In this communication we shall review the etiologic concepts of this disease and discuss the histologic features of several of our cases.

### ETIOLOGY

Theories as to the causation of ainhum are protean, but the etiologic factors comprised in them may be grouped under three general headings: infectious, constitutional and mechanical.

Zambaco pacha<sup>3</sup> was the outstanding proponent of the theory that ainhum was a manifestation of leprosy, but his views were vigorously opposed by Herrick and Earhart,<sup>4</sup> Darling (quoted by Herrick and Earhart<sup>4</sup>), Hektoen,<sup>5</sup> de Brun<sup>6</sup> and others.<sup>7</sup> The treponemes of yaws and syphilis were suggested as causes by Bharucha,<sup>8</sup> Wright<sup>9</sup> and Delanoë.<sup>10</sup> Other writers<sup>11</sup> have considered bacterial causation,

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1. Clark, cited by numerous writers.
2. da Silva Lima, J. F.: Gaz. med. da Bahia **1**:146, 1867; Arch. Dermat. **6**:367, 1880.
3. Zambaco pacha: Tr. Inst. Leprosy Conf. **3**:45, 1897.
4. Herrick, A. B., and Earhart, T. W.: Proc. Canal Zone M. A. **3**:26, 1910-1911.
5. Hektoen, cited by Herrick, J. B.: Philadelphia M. J. **1**:246, 1898.
6. de Brun, H.: Ann. de dermat. et syph. **10**:325, 1899.
- 7.(a) Acton, H. W.: Indian J. M. Research **15**:1085, 1928. (b) Friedman, L. J.: Am. J. Roentgenol. **31**:349, 1934.
8. Bharucha, E. S.: Indian M. Gaz. **52**:403, 1917.
9. Wright, L. T.: Urol. & Cutan. Rev. **28**:135, 1924.
10. Delanoë, L.: Bull. Soc. path. exot. **18**:470, 1925.
11. (a) Shepherd, F. J.: Am. J. M. Sc. **93**:137, 1887. (b) Horwitz, O.: Med. & Surg. Reporter **56**:649, 1887.

Shepherd even predicting that an "ainhum bacillus" might be found which would produce a comparable disease in mice and rabbits, provided the animals inoculated were not white! Davies and Hewer<sup>12</sup> stated that epidermophytosis might produce ainhum, but neither they nor Dschang<sup>13</sup> was able to demonstrate fungi. Parasitologic theories have been advanced, and Wellman<sup>14</sup> wrote at length on the possible role of *Sarcopsylla penetrans* as a causative factor. Recently Martens and Norris<sup>15</sup> suggested that chronic osteomyelitis may be responsible for the development of ainhum.

Of the constitutional causes, trophoneurosis, that is "some trophic disturbance of the nerve centers,"<sup>11a</sup> has appeared most frequently in the literature on this subject.<sup>16</sup> McKnight<sup>17</sup> performed lumbar sympathetic ganglionectomy in a patient with ainhum; when healing occurred he postulated that overactivity of the sympathetic nerves produced vascular spasm resulting in ainhum. Guimarães<sup>18</sup> stated that the immediate cause was contracture of the arteries but did not specify the basis of that. Anatomic peculiarities of the toes of Negroes have been pointed out as predisposing to vascular embarrassment.<sup>19</sup> Matas,<sup>16e</sup> in considering trophoneurosis as a possible cause, stated that the end result might well be annular scleroderma; others have agreed.<sup>20</sup> Evidence purporting to show that ainhum is but a peculiar manifestation of hyperkeratosis plantare et palmarum has been well reviewed by Spencer<sup>21</sup> and ably criticized by Spinzig.<sup>22</sup> The latter held, as do we, that the term "ainhum" is applicable only when the specific lesion of the toe occurs alone and that ainhum should not "be considered a symptom when constrictions occur in association with other diseases. . . . The latter type of constriction may be called 'ainhum-like.'"

Only one reference suggesting dysfunction of the endocrine glands as an etiologic factor was found.<sup>7a</sup>

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12. Davies, J. N. P., and Hewer, T. F.: Tr. Roy. Soc. Trop. Med. & Hyg. **35**:125, 1941.

13. Dschang, Y. D.: Virchows Arch. f. path. Anat. **290**:648, 1933.

14. Wellman, F. C.: J. Trop. Med. **9**:31, 1906; **11**:117, 1908.

15. Martens, V. E., and Norris, R. F.: U. S. Nav. M. Bull. **45**:745, 1945.

16. (a) Weinstein, H.: Proc. Canal Zone M. A. **4**:110, 1911-1912. (b) Welch, R. S. G.: U. S. Nav. M. Bull. **21**:352, 1924. (c) Brayton, N. D.: J. A. M. A. **45**:87, 1905. (d) Abbe, T.: M. Rec. **79**:478, 1911. (e) Matas, R.: New Orleans M. & S. J. **16**:603, 1888-1889; Tr. Am. S. A. **14**:483, 1896.

17. McKnight, R. B.: North Carolina M. J. **1**:76, 1940.

18. Guimarães, cited by Dell'Orto, J.: New Orleans M. & S. J. **8**:516, 1880-1881.

19. Ashley-Emile, L. E.: J. Trop. Med. **8**:33, 1905.

20. Messum, G.: Lancet **1**:932, 1891. McKnight.<sup>17</sup> Guimarães.<sup>18</sup> Ashley-Emile.<sup>19</sup>

21. Spencer, H. A.: South African M. Rec. **5**:103, 1907.

22. Spinzig, E. W.: Am. J. Roentgenol. **42**:246, 1939.

That the disease is familial is accepted by many. Horwitz and Tunick,<sup>23</sup> Duhring<sup>24</sup> and others<sup>25</sup> have observed the lesion in several members of the same family. Relationship to other Negro tendencies, such as that toward keloids, has not been definitely established, but several investigators considered that ainhum may bear some relation to the "fibrogenetic" tendency of the race.<sup>26</sup> Despite a statement to the contrary,<sup>16c</sup> in utero development of ainhum has not been observed; congenital spontaneous amputations should not be confused with ainhum.

The final group of theories concerns mechanical factors. The little toe, because of its position, may be especially liable to injury,<sup>27</sup> to minute abrasions by jungle grasses and the like. As was indicated in a clinical communication,<sup>28</sup> this can hardly be the sole explanation. On the Isthmus of Panama the 45 patients exhibiting ainhum were exclusively Negroes, whereas Panamanian mestizos, who wear shoes with even less frequency than the Negroes, seemed to be free of the disease. The wearing of toe rings or the application of ligatures is not responsible although, as Wile pointed out, the appearance of the lesions strongly suggests such mechanical causes.<sup>24</sup>

#### PATHOLOGY

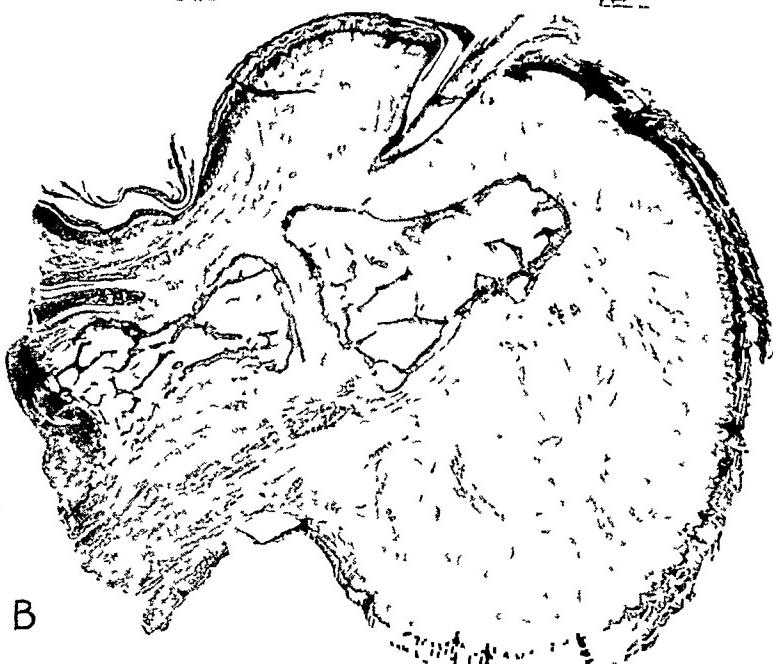
The first studies on the pathologic anatomy of ainhum were made by Wücherer<sup>28</sup> at the request of da Sillva Lima, in Bahia, Brazil. The former sent material to Weber in London, England, where De Morgan and Wood first reported in 1867,<sup>25b</sup> and to von Schüppel, of Tübingen, Germany, who published a report in 1872.<sup>29</sup> Following these early reports, many excellent descriptive studies have appeared,<sup>30</sup> but little new has been added.

During the past several years we have had the opportunity to review the records of 45 persons suffering from ainhum on the Isthmus of Panama and to observe a dozen clinically. We have been able to study histologic material in 6 cases, 1 of which is illustrated in the accompanying figures. The descriptions based on these data conform to a considerable degree with previous reports, but our interpretations may be at some variance with those of other workers.

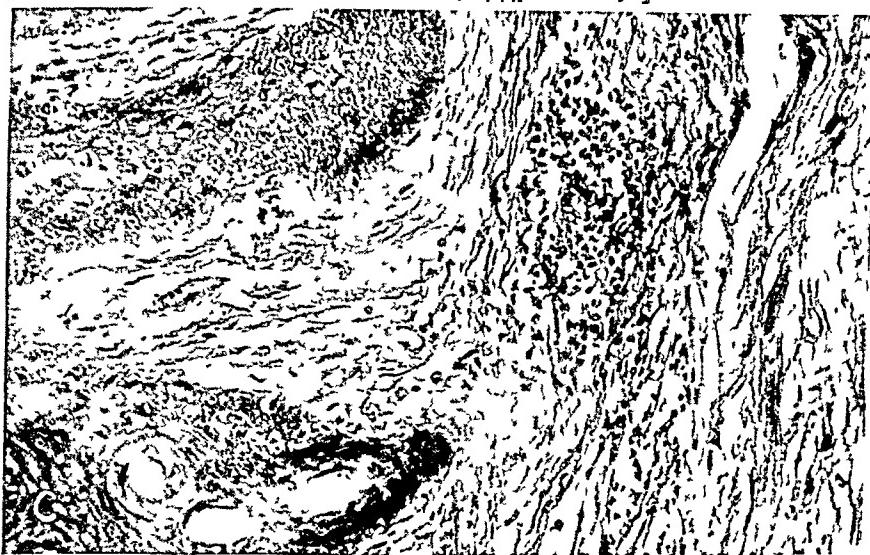
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- 23. Horwitz, M. T., and Tunick, I.: Arch. Dermat. & Syph. **36**:1058, 1937.
  - 24. Duhring, L. A.: Am. J. M. Sc. **87**:150, 1884.
  - 25. (a) Dupouy, E.: Arch. de méd. nav. **41**:260, 1884. (b) Weber, H.: Tr. Path. Soc. London **18**:277, 1867. (c) Wheatland, M. F.: J. A. M. A. **45**:631, 1905. (d) Doyle, E. A. G.: Brit. M. J. **1**:1346, 1889. (e) Simon, K. M. B.: J. A. M. A. **76**:590, 1921. (f) Weinstein.<sup>16a</sup>
  - 26. Kean, B. H.; Tucker, H. A., and Miller, W. C.: Tr. Roy. Soc. Trop. Med. & Hyg. **39**:331, 1946.
  - 27. Eyles, C. H.: Lancet **2**:576, 1886.
  - 28. Wücherer, O.: Virchows Arch. f. path. Anat. **56**:374, 1872.
  - 29. von Schüppel, F. G.: Virchows Arch. f. path. Anat. **56**:381, 1872.
  - 30. Davies and Hewer.<sup>12</sup> Dschang.<sup>13</sup> Weinstein.<sup>16a</sup> Duhring.<sup>24</sup> Eyles.<sup>27</sup>



A



B



Case 1. *A*, bisected toe with ainhum showing the typical "groove"; a moderately advanced lesion;  $\times 2$ . *B*, microtessar photograph of a section of the toe with ainhum;  $\times 4$ . Periostitis of significant degree was not seen microscopically. *C*, low grade inflammatory changes present in the corium and the subcutaneous tissues;  $\times 125$ . These changes seemed to be no more marked at the site of the furrow than in other portions of the sections studied. (Mr. Julius Weber, Department of Photography, Columbia University College of Physicians and Surgeons, took photograph designated *C*.)

The disease in our material was limited to Negro males, and the little toe was the only digit involved. At the level of the digitoplantar fold was a deep encircling groove or furrow separating the toe from the remainder of the tarsus. The toe itself was bulbous, often swollen and frequently painful. The nail was atrophied. When advanced, the "constriction" had progressed until a thin pedicle kept the small potato-like toe attached to the foot. (Excellent photographs of the lesion are available in most of the standard textbooks of tropical medicine.) In our last 3 cases the toe was amputated *proximal* to the groove so that the site of "constriction" could be adequately studied.

In microscopic sections the epithelium of the toe showed moderately severe hyperkeratosis, some parakeratosis and slight to moderate irregular acanthosis. Surprisingly enough, however, these changes were only slightly more intense at the site of the groove than either distally or proximally. Within the superficial and the deeper layers of the corium and in the subcutaneous tissue was a slight to moderate chronic inflammatory reaction with infiltrating lymphocytes and plasma cells (*C* in figure). Occasionally neutrophils and giant cells of the chronic inflammatory reaction type could be seen. Fibrosis generally was moderate and assumed no pattern which conceivably could cause the "constricting band" which has figured so conspicuously in some previous descriptions.<sup>31</sup> In some areas the fibrous tissue was hyalinized; in others it appeared fragmented. The bone was atrophied and had generally disappeared at the site of the groove. Osteoclasts could occasionally be identified. The walls of the blood vessels were thick and the lumens narrow, and infrequently the intimal layer showed proliferation. The nerves on the whole were not remarkable, although some perineural fibrosis could be identified. The glands generally appeared normal, although often widely separated by fibrous tissue. Much fat was generally present in the subcutaneous tissue of the ventral aspect of the toe.

The foregoing description conforms rather monotonously to the many previously published reports on ainhum. However, we believe undue significance has been placed on various histologic features:

- (a) The groove or furrow at the digitoplantar fold is not caused by a *constricting band* of fibrous tissue.
- (b) The epithelial changes are not striking and identical changes may be seen in the toes of those who do not have ainhum.
- (c) The thick walls of the blood vessels and the occasionally proliferated intima are matched in controls.
- (d) The changes in nerves and glands do not appear significant.

31. Tye, M.: New England J. Med. **234**:152, 1946. Gray and Blache, cited by Spinzig.<sup>22</sup> Acton.<sup>7a</sup> Friedman.<sup>7b</sup> Davies and Hewer.<sup>12</sup> Duhring.<sup>24</sup>

There remain the atrophy of bone and the subcutaneous inflammatory reaction and fibrosis, changes which seem significant but are difficult to evaluate. The changes in the bones have been of great interest for many years, especially from a roentgenologic standpoint.<sup>32</sup> Recently Martens and Norris,<sup>15</sup> reporting a case of ainhum, stated "the essential lesion appears to be a chronic osteomyelitis" and that "the fibrous constriction of the little toe is not the cause of bone destruction but is the result of cicatrization of previously inflamed tissue." Incidentally, they sectioned toes of patients without ainhum and noted thick-walled vessels like those often described as of significance in ainhum, a finding which is mentioned also by Ash and Spitz<sup>33</sup> and which we have noted in sections of the toes of our controls.

In our material the evidence that chronic osteomyelitis is the essential lesion of ainhum has not been impressive. More suggestive have been the low grade cellulitis and fibrosis of the corium. In 44 of the 45 patients on the Isthmus of Panama pain was a symptom sometime during the course of the disease. In several patients cellulitis was so prominent that amputation was delayed for fear of spreading the infection. From 2 patients recently observed, pure cultures of *Staphylococcus aureus* (hemolytic) were obtained when the area of "constriction" was incised and pus released. It is, of course, possible that this infection may be secondary, occurring in a locus minoris resistentiae. In sections available for study, however, the inflammatory reaction is not extensive, and it is difficult to conceive how it could produce the lesion which is so distinctive grossly and so difficult to define microscopically.

#### SUMMARY

In summary, the cause of ainhum is unknown, and its pathogenesis is not clear. The racial factor seems most important.

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Harold A. Tucker, The Johns Hopkins Hospital, Department of Medicine, Baltimore, Md.

32. Herrick and Earhart.<sup>4</sup> Friedman.<sup>7b</sup>

33. Ash, J. E., and Spitz, S.: *An Atlas of Pathology of Tropical Diseases*. Philadelphia, W. B. Saunders Company, 1945, p. 344.

# PRIMARY CYSTIC TUMOR OF THE DIAPHRAGM

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AND

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**T**HE first report of a primary tumor (fibroma) of the diaphragm was made by Granchi<sup>1</sup> in 1868. The paucity of similar reports since then indicates the rarity of primary diaphragmatic neoplasms. In 1944 Robson and Collis,<sup>2</sup> in summarizing the data on the recorded ones, were able to collect 21 benign and cancerous tumors primary in the diaphragm and added a benign one observed by them. In a recent review of this subject we have found a total of 32 reported benign and cancerous diaphragmatic tumors. This series includes 4 the diagnosis of which was not confirmed by operation or autopsy.<sup>3</sup> It is anticipated,

*TABLE 1.—Noncancerous Diaphragmatic Tumors*

Author	Year	Sex	Age	Side	Type	Symptoms
1. Granchi <sup>1</sup> .....	1868	M	..	..	Fibroma	Not described
2. Clarke <sup>2a</sup> .....	1887	F	65	R	Lipoma	None
3. Kramer <sup>11</sup> .....	1899	M	54	R	Chondroma	None
4. Bonamy <sup>10a</sup> .....	1912	F	34	R	Multiple fibroma	Swelling; no pain
5. Burvill-Holmes and Brody <sup>12</sup> .....	1932	M	73	R	Angiosfibroma	None
6. Spangenberg, Gattini and Sloer <sup>3c</sup> .....	1935	M	73	R	Unknown	Not stated
7. Gravano <sup>3b</sup> .....	1935	..	..	..	Unknown	.....
8. Soderlund <sup>2b</sup> .....	1937	F	50	L	Lipoma	Pain in chest
9. Ballon and Spector <sup>9c</sup> .....	1939	F	45	L	Lipoma	None
10. Kinsella <sup>10</sup> .....	1939	F	62	R	Fibromyoma	Mass between ribs; unrelated pain
11. Butler <sup>17a</sup> .....	1939	F	34	L	Cyst (type?)	Pain in chest
12. Nylander <sup>13</sup> .....	1942	M	17	R	Lymphangioma	Abdominal pain
13. Arkless <sup>16</sup> .....	1942	M	38	L	Rhabdomyosfibroma	Pain in chest; cough
14. Denis <sup>3a</sup> .....	1942	F	20	R	Unknown	Pain in chest
15. Ackerman <sup>3d</sup> .....	1942	M	48	R	Unknown	Pain in chest
16. Soto <sup>2d</sup> .....	1943	M	14	L	Lipoma	Pain in chest
17. Robson and Collis <sup>2</sup> .....	1944	M	22	R	Cyst (type?)	Pain in chest; dyspnea
18. Klassen, Patton and Bemen <sup>14</sup> .....	1945	M	42	R	Neurofibroma	Pain in chest
19. Scott and Morton..... (Present report)	1946	M	29	L	Cyst (type?)	Pain in chest

From the Department of Surgery, University of Chicago Clinics.

This study was facilitated by a grant from Mr. Hill Blackett, Chicago.

1. Granchi, M.: Bull. Soc. anat. de Paris **43**:385, 1868.

2. Robson, K., and Collis, J. L.: Brit. J. Tuberc. **38**:3, 1944.

3. (a) Denis, R., and Garre, O.: Prensa méd. argent. **29**:380, 1942. (b)

Gravano, L.: Semana médica. **2**:705, 1935. (c) Spangenberg, J. J.; Gattini, H., and Sloer, M.: Prensa médica argentina. **22**:17, 1935. (d) Ackerman, H. J.: Am. J. Roentgenol. **47**:711, 1942.

however, that with the increasing use of routine fluoroscopy and roentgenography of the chest diaphragmatic tumors will be found with increasing frequency. Reports of tumors are summarized in tables 1 and 2, including the one reported here.

#### REPORT OF TUMOR

G. H., a 29 year old white man, was first admitted to the University of Chicago Clinics (service of Dr. A. Brunschwig) on Aug. 19, 1945, at which time he stated that two years previously he had consulted a physician because of poor appetite, loss of weight and fatigue. At that time a diagnosis of pulmonary tuberculosis was made on the basis of roentgenograms of the chest and "positive sputum."

TABLE 2.—*Primary Diaphragmatic Cancers*

Author	Year	Sex	Age	Side	Type	Symptoms
1. Dalzell <sup>4a</sup> .....	1887	F	42	R	Round cell sarcoma	
2. Alexander <sup>4a</sup> .....	1896	..	..	..	Fibrosarcoma	Cough
3. Gross <sup>4b</sup> .....	1911	M	30	L	Fibrosarcoma	None described
4. Sauerbruch <sup>4a</sup> .....	1913	F	43	L	Fibromyosarcoma	Pain in chest
5. Van Nes <sup>4c</sup> .....	1921	F	65	L	Fibrosarcoma	Abdominal pain
6. Muller <sup>5b</sup> .....	1933	F	45	R	Myosarcoma	Abdominal pain
7. Kirschbaum <sup>5c</sup> .....	1935	M	58	L	Leiomoyosarcoma	None
8. Kirschbaum <sup>5e</sup> .....	1935	M	47	R	Rhabdomyosarcoma	Palpable mass
9. Ryan <sup>5d</sup> .....	1939	..	..	..	Myosarcoma	Thoracic pain, hemoptysis
10. Peery and Smith <sup>5e</sup> .....	1939	M	44	R	Rhabdomyosarcoma	None described
11. Gale and Edwards <sup>8</sup> .....	1939	M	54	R	Carcinoma of liver from embryonal rest	Pain in chest; dyspnea
12. Petacci <sup>7</sup> .....	1940	M	45	R	Undifferentiated sarcoma	Pain in shoulder
13. Hyman and Lederer <sup>4d</sup> ...	1941	F	73	R	Fibrosarcoma	Pain in chest
14. Ackerman <sup>3d</sup> .....	1942	M	27	R	Fibrosarcoma	Edema of legs Pain; cough

Eight subsequent examinations of his sputum gave negative results. Subsequently he spent three weeks in a sanatorium, where after a thorough examination he was informed that he did not have tuberculosis but a cyst of the left lung. At about this time he began to note a sharp, nonradiating pain at the left costal margin on deep inspiration or on bending or lifting.

For two or three years prior to his admission he noted a mild productive cough, but the sputum was not purulent, and no hemoptysis occurred. So far as he knew he had not been in contact with a tuberculous person. Although his weight fluctuated considerably, he was usually underweight, and one month before admission he was rejected for military duty because he was "underweight and neurotic."

On admission the examination gave essentially negative results. The blood hemoglobin content was 16.2 Gm.; the red blood cell count, 4,590,000; the white cell count, 7,800; the Kahn test, negative. There were no abnormal urinary findings. Roentgenograms of the chest revealed, near the costophrenic angle, a rounded protrusion from the left leaf of the diaphragm, which measured 3 by 6 cm. in diameter (fig. 1A). Along the inferior medial aspect of the mass a shell of calcification was noted. At fluoroscopy the rounded mass was seen to move freely with the left leaf of the diaphragm. Roentgenograms of the stomach and

duodenum showed no relationship between these organs and the mass in the left side of the diaphragm. The Casoni test (intradermal injection of echinococcus antigen) was negative.



Fig. 1.—A, roentgenogram of the lower part of the chest showing a mass in the left leaf of the diaphragm with a calcified medial wall. B, intact specimen showing (a) the pleural surface above, (b) the peritoneal surface below and (c) the surrounding diaphragmatic muscle.

1 as neurofibroma,<sup>14</sup> 1 as rhabdomyofibroma,<sup>15</sup> 1 as fibromyoma<sup>16</sup> and 3 as cysts.<sup>17</sup> Four tumors in regard to which a final diagnosis of diaphragmatic tumor could not be made are included in the tabulation of benign tumors.<sup>3</sup>

These neoplasms were found in patients varying from 14 to 73 years of age, with an average age of 43. Of the 28 tumors whose locations were stated, 18 were in the right leaf and 10 were in the left leaf of the diaphragm. Eighteen of the tumors were in males and 12 in females. With regard to 2 the sex of the patient was not stated.

Primarily diaphragmatic tumors did not always produce symptoms, and when the latter were present they followed no specific pattern. The most constant symptom noted was pain in the chest, also in the hypochondrium and occasionally radiating along the course of the regional intercostal nerves or to the shoulder. Cough was occasionally a complaint and hemoptysis once. Bulging intercostal masses, as well as masses in the right and left upper quadrants of the abdomen have been noted. Dyspnea is sometimes present, and pleural effusion may occur.

The clinical diagnosis can hardly be made on the basis of the history and the results of the physical examination, owing to the rarity of the condition; it is finally made on roentgenographic evidence, and it is possible to differentiate between abdominal and intrathoracic masses by roentgen study.<sup>3d</sup> Intrathoracic tumors touching the diaphragm show motion synchronous with the normal respiratory excursion of the diaphragm. If the tumor lies underneath the diaphragm and protrudes through it into the thoracic cavity, a paradoxical movement of the tumor's shadow is noted similar to that characteristic of diaphragmatic hernia. The paradoxical movement of a diaphragmatic hernia may not be demonstrable in the presence of adhesions within the hernial contents. Diagnostic pneumothorax is useful in differentiating lesions occurring in the base of the lung. Pneumoperitoneum may aid in outlining the position of the tumor. Roentgenographic examination of the gastrointestinal tract is of value in differentiating lesions involving the stomach or the bowel. When cystic tumors with calcified walls are encountered, intra-dermal or complement fixation tests are indicated to rule out echinococcus cysts.

14. Klassen, K. P.; Patton, R., and Bemen, F.: J. Thoracic Surg. **14**:407, 1945.

15. Arkless, H. A.: M. Bull. Vet. Admin. **19**:225, 1942.

16. Kinsella, T. J., in discussion on Gale and Edwards.<sup>8</sup>

17. (a) Butler, E. F., in discussion on Gale and Edwards. <sup>8</sup> (b) Robson and Collis.<sup>2</sup>

# DEVELOPMENT OF SEBACEOUS GLANDS FROM INTRALOBULAR DUCTS OF THE PAROTID GLAND

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AS GRUENWALD has recently pointed out, the lability of determination and presence of latent potencies of cells are more common than was originally believed, and morphologic determinations which were thought to be fixed could be changed under experimental conditions, such as tissue culture and regeneration.<sup>1</sup> It is difficult, however, to obtain an accurate knowledge of the different prospective potencies of the cells of the various tissues, as the finding of the appropriate developmental stimulus for obtaining certain differentiations depends more or less on chance. In human pathology, accidental findings may sometimes bring to light unsuspected potencies of certain cells or prove the reality of potencies whose presence had been assumed on theoretic grounds. Of this the following case is a good example.

## REPORT OF A CASE

A white woman 34 years old had been operated on elsewhere for a tumor of the left parotid gland. Examination showed it to be a noncancerous mixed tumor. There was a local recurrence of the growth, slowly growing, which was removed with the surrounding glandular tissue (Dr. M. J. Hugenholtz). At this moment, two and a half years after operation, the patient is without recurrence.

Microscopically, the tumor was a typical mixed tumor of the parotid gland, consisting of strands and nests of small epithelial cells with thin intercellular bridges and several glandular cavities, lying in a myxomatous stroma which in several places resembled cartilage. There was sometimes squamous metaplasia with cornification. In the stroma lay large cells with long, irregular processes. Only after a prolonged search could an isolated mitosis be found in the tumor cells. The tumor was generally well encapsulated. In a few places, however, there was some infiltration of the capsule by tumor tissue. The lobules of the parotid gland immediately bordering on the tumor capsule showed atrophy and partial disappearance of the acini and some lymphocytic infiltration. There were numerous intercalated ducts, the protoplasm of which sometimes contained small granules staining with aniline blue. Here and there a mitotic division was observed in the ductal epithelium.

The other lobules showed the normal structure of the parotid gland. The cells of the acini contained a moderate number of secretory granules, and each had a narrow rim of chromophil substance. In general, the striated and inter-

1. Gruenwald, P.: Arch. Path. 36:190, 1943.

1 as neurofibroma,<sup>14</sup> 1 as rhabdomyofibroma,<sup>15</sup> 1 as fibromyoma<sup>16</sup> and 3 as cysts.<sup>17</sup> Four tumors in regard to which a final diagnosis of diaphragmatic tumor could not be made are included in the tabulation of benign tumors.<sup>3</sup>

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AS GRUENWALD has recently pointed out, the lability of determination and presence of latent potencies of cells are more common than was originally believed, and morphologic determinations which were thought to be fixed could be changed under experimental conditions, such as tissue culture and regeneration.<sup>1</sup> It is difficult, however, to obtain an accurate knowledge of the different prospective potencies of the cells of the various tissues, as the finding of the appropriate developmental stimulus for obtaining certain differentiations depends more or less on chance. In human pathology, accidental findings may sometimes bring to light unsuspected potencies of certain cells or prove the reality of potencies whose presence had been assumed on theoretic grounds. Of this the following case is a good example.

### REPORT OF A CASE

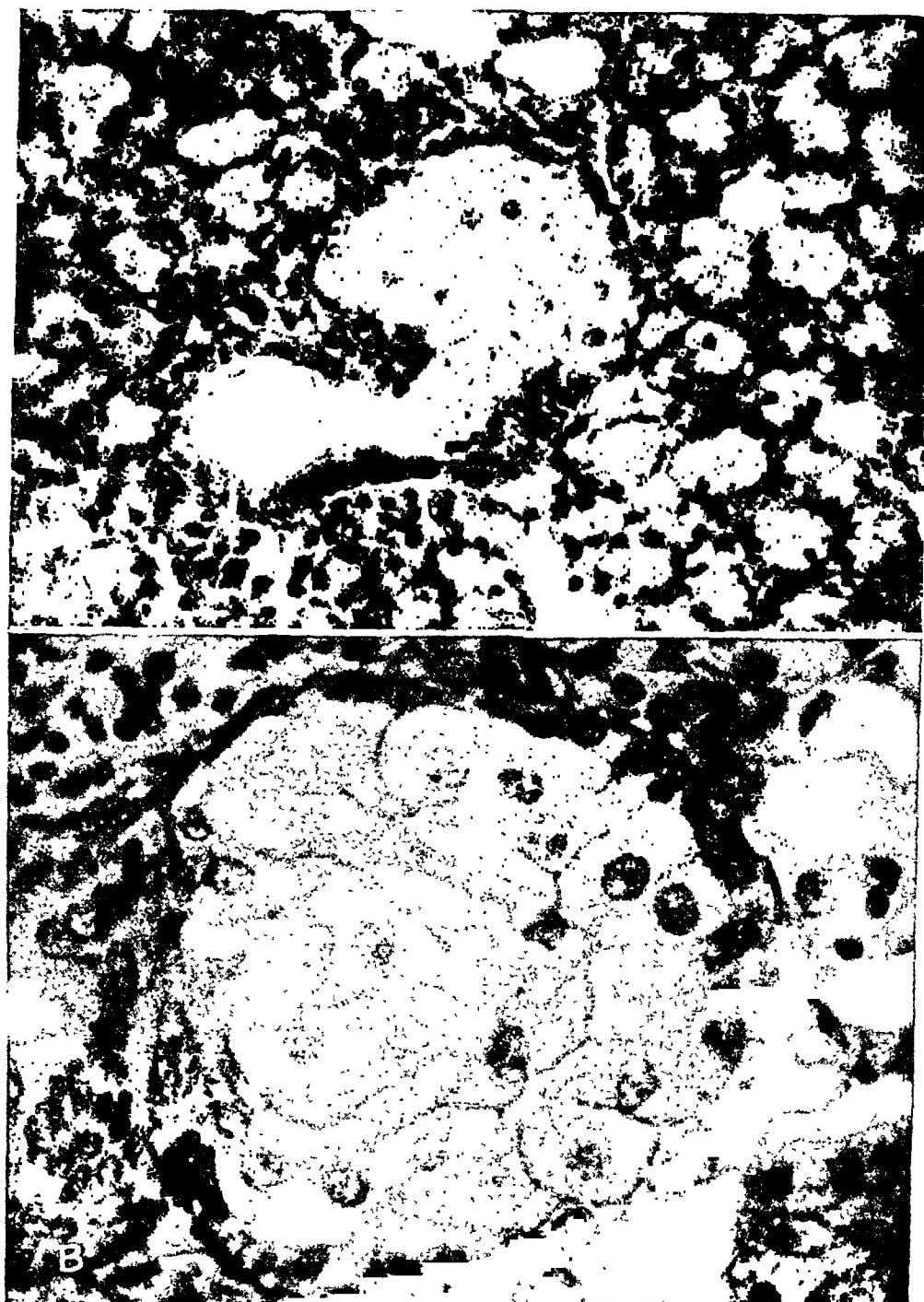
A white woman 34 years old had been operated on elsewhere for a tumor of the left parotid gland. Examination showed it to be a noncancerous mixed tumor. There was a local recurrence of the growth, slowly growing, which was removed with the surrounding glandular tissue (Dr. M. J. Hugenholtz). At this moment, two and a half years after operation, the patient is without recurrence.

Microscopically, the tumor was a typical mixed tumor of the parotid gland, consisting of strands and nests of small epithelial cells with thin intercellular bridges and several glandular cavities, lying in a myxomatous stroma which in several places resembled cartilage. There was sometimes squamous metaplasia with cornification. In the stroma lay large cells with long, irregular processes. Only after a prolonged search could an isolated mitosis be found in the tumor cells. The tumor was generally well encapsulated. In a few places, however, there was some infiltration of the capsule by tumor tissue. The lobules of the parotid gland immediately bordering on the tumor capsule showed atrophy and partial disappearance of the acini and some lymphocytic infiltration. There were numerous intercalated ducts, the protoplasm of which sometimes contained small granules staining with aniline blue. Here and there a mitotic division was observed in the ductal epithelium.

The other lobules showed the normal structure of the parotid gland. The cells of the acini contained a moderate number of secretory granules, and each had a narrow rim of chromophil substance. In general, the striated and inter-

1. Gruenwald, P.: Arch. Path. 36:190, 1943.

calated ducts showed nothing particular except a few small ducts lined by mucous cells. In several otherwise normal lobules there were epithelial structures which did not belong to the normal components of the parotid gland. They varied



*A*, sebaceous gland developing from a small duct;  $\times 280$ . There is holocrine secretion. *B*, higher magnification to show the flattened cells at the periphery and the large nuclei of the sebaceous gland;  $\times 580$ .

much in size; the diameter of the larger ones was as much as 400 microns. They were surrounded by a thin basement membrane. They were partly solid and consisted of epithelial cells, the outermost layer being flattened, with fairly dark-staining protoplasm, whereas the more centrally placed cells contained protoplasm which showed fine progressive vacuolation. It did not contain chromophil substance or secretory granules. The nuclei were larger than those of the acinous cells; they contained less chromatin and a distinct nucleolus. In the central cells the nuclei were sometimes irregularly indented by the vacuoles and pyknotic.

Serial sections showed that these structures always originated from ducts, either striated or intercalated ducts. The epithelium of the ducts transformed into flattened cells became multilayered and gave origin to the structures described. The transformation of the ductal epithelium was fairly sudden. The impression was gained that the abnormal epithelial formations did not develop in the continuity of the ducts but in short branches which ended blindly. There was sometimes typical holocrine secretion. Mitotic divisions were only rarely observed.

#### COMMENT

It follows from the foregoing description and the photomicrographs that the epithelial formations could not be distinguished from small sebaceous glands. By their nuclear structure, by the progressive vacuolation of their cytoplasm and by the absence of chromophil substance and secretory granules they could easily be differentiated from the acinous cells of the parotid gland.

It could be proved that the sebaceous glands developed from intralobular ducts, striated or intercalated. Unfortunately, it was impossible to determine when this development had started, so that it remains unknown whether they were present before the tumor and the first operation or whether these abnormal circumstances were the stimulus for the peculiar differentiation of the ductal epithelium.

Theoretically, the development of sebaceous glands from the ducts of the parotid gland cannot be considered as strange, though the literature disclosed no instance and only 1 case of mixed tumor of the parotid gland containing sebaceous glands.<sup>2</sup> The parotid gland develops from the ectoderm on the deep aspect of the cheek,<sup>3</sup> and it is also known that during puberty sebaceous glands develop in this region.<sup>4</sup> The case

2. Harvey, W. F.; Dawson, E. K., and Innes, J. R. M.: *Debatable Tumours in Human and Animal Pathology*, London, Oliver & Boyd, Ltd., 1940, p. 24, fig. 25.

3. Hamilton, W. J.; Boyd, J. D., and Mossman, H. W.: *Human Embryology*, Baltimore, Williams & Wilkins Company, 1945, p. 161.

4. Weatherford, H. L.: *A Textbook of Histology*, Philadelphia, The Blakiston Company, 1944, p. 319.

which I have described shows therefore a prospective potency of the ductal epithelium of the parotid gland which could be presumed on theoretic grounds.

#### SUMMARY

In the normal lobules of a parotid gland showing a recurrence of a typical mixed tumor were found small sebaceous glands developing from striated or intercalated ducts.

## Case Reports

### LEIOMYOSARCOMA INVOLVING THE RIGHT URETER

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LEIOMYOSARCOMA is not a common tumor. There seems to be no distinct characteristic clinical picture, so that the final diagnosis must await microscopic study. The tumor is highly vascularized, has little stroma and is studded with mitotic figures. Its cells are spindle-shaped, unstriated muscle type with large deeply staining nuclei. It seems that any organ having unstriated muscle fibers may become the site of development of leiomyosarcoma. In looking over the literature we have been unable to find a report of a case of leiomyosarcoma similar in site to the one to be recorded later in this presentation.

Krauskopf<sup>1</sup> reported an analysis of 30 cases, to which he added his own. The sites were as follows: pleura, foot and vulva (1 case each); urinary tract (5 cases—the growth involving the bladder in 3 and the kidneys in 2); gastrointestinal tract (7—the growth involving the cecum, the ileum and the liver in 1 case each and the stomach and the jejunum in 2 cases each); uterus (16 cases). In a review of the literature on smooth muscle tumors of the stomach Chaffin<sup>2</sup> collected 363 cases, 16 of which were definitely cases of leiomyosarcoma. Concerning 2 of the 16, no data were recorded as to sex and age, and therefore these 2 could not be used in our table 1. Since the publication of Chaffin's review Baumgartner<sup>3</sup> has reported 1 case and Horsley and Berger<sup>4</sup> 3 cases of leiomyosarcoma of the stomach. Hallock, Watson and Berman<sup>5</sup> reported an interesting case in which the tumor arose from the subdiaphragmatic portion of the inferior vena cava. To this group we add a case in which retroperitoneal leiomyosarcoma was densely adherent to the right uterter.

Table 1 reveals that the frequently heard statement that sarcoma occurs principally in the young and carcinoma in the middle-aged and the aged is not valid so far as leiomyosarcoma is concerned. It might best be said that cancer is a disease principally of middle life but may occur at any period of life. In regard to sex incidence, table 1 cannot be used as a criterion, because included in it are 16 cases of uterine and 1 case of vulvar leiomyosarcoma. Table 2 provides a

1. Krauskopf, H.: Am. J. Surg. 22:192, 1933.
2. Chaffin, L.: West. J. Surg. 46:513, 1938.
3. Baumgartner, C. J.: West. J. Surg. 47:27, 1939.
4. Horsley, G. D., and Berger, R. A.: Ann. Surg. 112:22, 1940.
5. Hallock, P.: Watson, C. J., and Berman, L.: Arch. Int. Med. 66:50, 1940.

better basis for judgment since only cases of leiomyosarcoma of the stomach are included. It will be noted that 65 per cent of the patients were females and 35 per cent were males. This is a ratio of almost 2:1 favoring females. It is contrary to the report by Horsley and Berger,<sup>4</sup> who stated: "There are no sex differences, the occurrence being about equal in males and females." Andersen and Doob,<sup>6</sup> while reporting a

TABLE 1.—*Sex and Age Incidence of Leiomyosarcoma*

Authors	Sex and Age, Yr.	
	Female	Male
Baumgartner <sup>3</sup> .....		39
Hallock and others <sup>5</sup> .....	31	..
Horsley and Berger <sup>4</sup> .....	62	..
	48	..
Chaffin <sup>2</sup> .....		34
	47	..
	43	..
	17	..
	63	..
	56	..
	65	..
	40	..
	32	..
	32	..
	34	..
		49
		40
		41
		33
Krauskopf <sup>1</sup> .....		..
	53	..
	60	..
	26	..
	40	..
	47	..
	44	..
	47	..
	46	..
	50	..
	38	..
	52	..
	55	..
	55	..
	40	..
	31	..
	29	..
	45	..
	36	..
	51	..
	21	..
	50	..
	50	..
	8	..
	48	..
	46	..
	..	4
	..	65
	..	50
	..	19
	..	35
	..	40
Rossien and Russell: Arch. Path., this issue.....	55	..
Number of each sex.....	59	12
Average age.....	41.23	36.55
Age span.....	17 to 83	4 to 65

case of leiomyosarcoma of the duodenum, mentioned 18 previously reported similar cases. They stated that, "of the 18 patients whose sex was specified, 12 were men." Their patient was also a male. Hence, their findings would, off hand, appear to be a reversal of our conclusion. However, the 5 cases of leiomyosarcoma of the urinary

6. Andersen, D. H., and Doob, E. F.: Arch. Path. 10:795, 1933.

tract included in Krauskopf's<sup>1</sup> report were distributed between the sexes in a ratio of 3:2 favoring females. Further, deducting from table 1 the 17 cases in which the female genitals were involved, one still notes that 64.7 per cent of the patients with leiomyosarcoma are females and 35.3 per cent are males.

#### REPORT OF CASE

Sept. 9, 1941 a white housewife aged 55 years reported for study and gave the following history:

About three months before, a burning sensation developed in the right lower abdominal quadrant and in the loin. At the onset the pain occurred immediately after meals and at three to four hour intervals. However, after a few weeks this

TABLE 2.—*Sex and Age Incidence of Leiomyosarcoma of the Stomach*

Authors	Sex and Age, Yr.	
	Female	Male
Baumgartner <sup>3</sup> .....	..	39
Horsley and Berger <sup>4</sup> .....	62	..
	48	34
Chaffin <sup>2</sup> .....	47	..
	43	..
	17	..
	68	..
	56	..
	65	..
	40	..
	32	..
	32	..
	34	..
	..	39
	..	40
	..	41
	..	33
	21	..
Krauskopf <sup>1</sup> .....	..	50
Number of each sex.....	13	7
Average age.....	43.46	39.42
Age span.....	17 to 68	33 to 50

burning pain, which at no time radiated, became constant. She was annoyed by large nonbleeding hemorrhoids and by gas in the abdomen. She had lost about 10 pounds (4.5 Kg.) in the past three months "which might have been due to eating only liquids because the pain became worse if solids were eaten." She was nervous and tired all the time. There were no symptoms referable to the head, the neck, the lungs, the extremities, the heart or the urinary system.

Her appetite was good. She was a rapid eater, with proper mastication. There was no difficulty on deglutition. The capacity of the stomach seemed less. There was no belching, heartburn or vomiting. Nausea developed during and after meals. There was hunger-like pain one hour after eating, which may have been due to the fact that the meals ingested were small. The patient never was jaundiced. She was intolerant toward bread and, owing to resultant abdominal pain, was afraid to eat any solid food. The pain of the right lower quadrant of the abdomen did not radiate. There had been some pain intermittently in the left lower quadrant and left loin for the past few months, since hemorrhoids were noticed protruding on defecation. The bowel movements were regular without medication. The stools were watery; no other gross changes were noted. Recently there had been some pain on defecation.

In her childhood the patient had whooping cough and attacks of sore throat. Twenty-five years prior to examination she had pleurisy with effusion. Twenty-three years before, she underwent appendectomy and uterine suspension.

Her mother died of gastric carcinoma. One brother died of pneumonia. Her father, two brothers and three sisters were living and well. Several paternal relatives were suffering from allergic conditions. There was no history of tuberculosis or of diabetes mellitus.

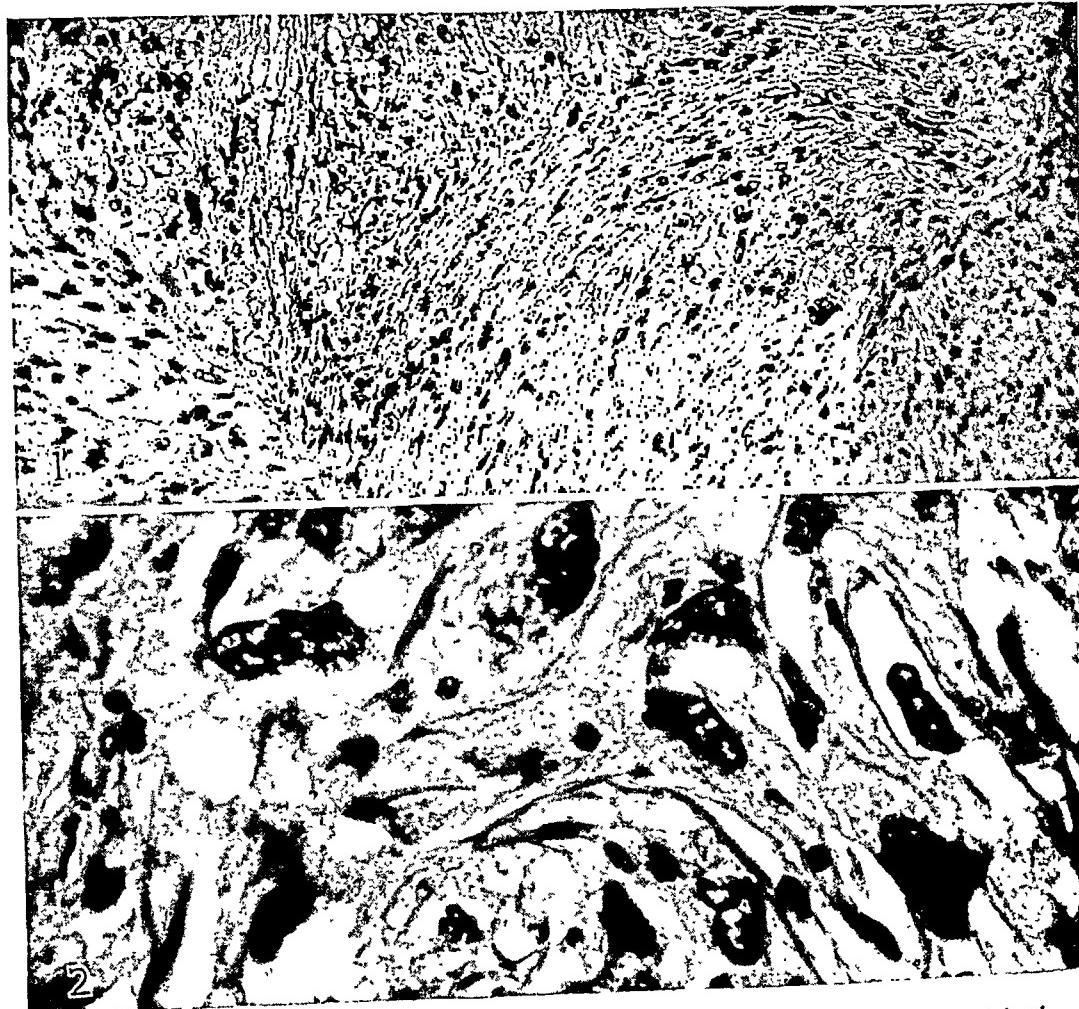


Fig. 1.—Leiomyosarcoma of ureter. Note whorls of tumor cells. These stained yellow by van Gieson's method, as do cells of smooth muscle origin.  $\times 150$ .

Fig. 2.—Leiomyosarcoma of ureter. Note the giant nuclei of the tumor cells, with bizarre shapes, coarse chromatin particles and large nucleoli. Compare with the nuclei of the slender connective tissue cells, of usual size, interspersed among the tumor cells.  $\times 675$ .

**Examination.**—The weight of the patient was  $141\frac{1}{4}$  pounds (64 Kg.). The blood pressure was 140 systolic and 90 diastolic. The pulse rate was 84; the pulse was regular and of good quality. The hemoglobin content was 68 per cent. The reflexes were hyperactive. There were fine tremors of the hands. There was slight prominence of the thyroid gland; no other glands were palpable.

Eyes, ears, nose, throat, teeth and gums presented nothing remarkable. There were no pathologic findings in the breasts. The heart had a presystolic murmur over the apex and a snappy second aortic sound. On fluoroscopy the left auriculoventricular angle was obliterated. In the right lower quadrant of the abdomen there was a mass the size of an orange, movable and tender. There was tenderness over the right upper and the left lower quadrant. Vaginal and rectal examinations presented nothing remarkable.

Cholecystographic study using a double dye method revealed chronic cholecystitis with cholesterol calculi. A flat plate study revealed gaseous distention of the colon with extrinsic pressure on the medial border of the cecum. A gastrointestinal roentgen series revealed duodenal stasis, six hour gastric retention of a small amount of ingested material, with visualization of portions of the small intestine, probably as a result of extrinsic pressure exerted by a mass in the abdomen, a probably retroperitoneal mass in the right lower quadrant. After a barium sulfate enema of the colon there was a shadow suggesting a mass in the right lower quadrant medial to the cecum and the ascending colon. This mass was thought to be retroperitoneal. Sigmoidoscopic examination revealed nothing remarkable apart from deduplication of the mucosal folds and internal hemorrhoids. The results of analyses of the stool, the urine and the gastric contents were essentially within normal limits.

The hemoglobin content was 68 per cent (Dare method); the red blood cell count, 4,295,000; the white cell count, 11,800 (neutrophils 53, small lymphocytes 39, large lymphocytes 4, basophils 3 and monocytes 1 per cent). The basal metabolic rate was plus 8.

*Operation.*—The patient was operated on at the New York Post-Graduate Medical School and Hospital by Dr. Thomas H. Russell, Oct. 23, 1941. A right paracostal incision was made and the abdominal cavity opened. The gallbladder was seen to be tremendously enlarged and edematous. It contained many stones and there was a stone impacted in the cystic duct. However, gallstones were not causing the patient's immediate trouble. A large retroperitoneal tumor was found on the inner side of the ascending colon at a point just to the right of the umbilicus. It was adherent to the peritoneum overlying it. A circular incision was made around the peritoneum so as to remove the diseased peritoneum with the tumor, and the tumor was carefully dissected upward. It was found that the right ureter was densely adherent to the posterior wall of the tumor. The ureter was dissected away from the tumor, and the tumor was removed. The ureter was dissected for a distance of fully 3 inches (7.5 cm.) and, as mentioned, a part of the tumor was left adherent to the ureter. Nephrectomy was considered on account of the adherence of the tumor to the ureter, but on examination the left kidney was found to be a cystic mass, and it was questionable as to how much work the organ was doing. Therefore, removal of the right kidney was out of the question. The liver was examined, and on its inner surface a small nodule the size of the end of the ring finger, looking just like the tumor, was found, which in all probability was metastatic. Frozen section showed the tumor to be a spindle cell sarcoma. The opening in the posterior layer of the peritoneum overlying the tumor was sutured with catgut. Because the patient's symptoms were partly due to obstructive cholecystitis, the gallbladder was opened, and a number of small and large stones were removed. The gallbladder was not removed but was drained by means of a no. 20 rectal tube. A cigaret drain was placed down to the foramen of Winslow, and the drain with the tube was brought out at the lower angle of the wound. The wound was closed with four through

and through silk sutures and in layers with catgut. The skin was closed with silk. The drainage tube was sutured to the skin with silk.

*Pathologist's Report.*—“Gross Examination: The first specimen consists of 50 faceted yellow friable stones with 3 ounces (88.5 cc.) of golden brown bile. The second specimen is a lobulated mass of pale gray resilient tissue partly covered by a thin capsule with attached yellow lobules of fat and measuring 9 by 5 by 4.5 cm. On section there is pale yellowish gray tissue, which is finely striated to form ill defined whorls. There are also some pale gray translucent areas several millimeters in diameter scattered at various points.

“Microscopic Examination: Paraffin-embedded sections show the following: Wide bands of spindle-shaped cells form an intertwining pattern with solid wide sheets of cells, and also weave a convoluted course through areas of necrosis. The cells vary in size, shape and staining properties. Simple spindle shapes are intermingled with thick short types, the latter often with giant and bizarre nuclei. Some are multinucleated, and most have rounded granular nuclei, varying greatly in size, and prominent nucleoli. Mitotic figures and bizarre and multinucleated forms are occasionally seen. With appropriate methods of staining the neoplastic spindle cells show myofibrils, by which they are identified as smooth muscle cells.

“Diagnosis: Gallstones; leiomyosarcoma.”

*Postoperative Course.*—The patient made an uneventful recovery from the operation. After an asymptomatic period of about three months there was a return of the pain in the right lower quadrant of the abdomen. The course thereafter was progressively downhill, and about two years after operation the patient died from generalized metastases.

#### SUMMARY

A leiomyosarcoma of the right ureter has been observed. No other cases of leiomyosarcoma of this site could be found recorded.

A tabulation of unselected cases of leiomyosarcoma reveals that the frequently repeated statement that sarcoma occurs principally in the young and carcinoma in the middle-aged and the aged is not valid so far as leiomyosarcoma is concerned.

The ratio of female to male patients is approximately 2:1 when one does not consider the incidence of uterine leiomyosarcoma. With that incidence included, the ratio would be closer to 3:1.

## PARATHYROID ADENOMA, WITH UREMIA DUE TO CALCIFICATION OF THE KIDNEYS

PAUL LOBER, M.D.; AMBROSE J. HERTZOG, M.D., and CARL O. RICE, M.D.  
MINNEAPOLIS

IT IS well known that primary hyperparathyroidism promotes the formation of renal calculi, but it is not so widely appreciated that it may injure the renal parenchyma and cause renal insufficiency. Cases of primary hyperparathyroidism associated with marked renal insufficiency have been reported by Elsom, Wood and Ravdin,<sup>1</sup> Baker and Howard<sup>2</sup> Bellin and Gershwin,<sup>3</sup> Albright, Baird, Cope and Bloomberg<sup>4</sup> and others. In some cases, such as that reported by Curtis and Feller,<sup>5</sup> it is difficult even at autopsy to determine whether the renal insufficiency preceded or followed the hyperparathyroidism. A review of the whole subject has been contributed by Anderson.<sup>6</sup>

The case reported here is of special interest because it was observed postoperatively for three years before death occurred from uremia. After the surgical removal of the parathyroid adenoma, renal insufficiency persisted from severe calcification of the kidneys. A state of secondary or compensatory hyperparathyroidism occurred as a result of the lowering of the serum calcium and the elevation of the serum phosphorus. It closely resembles the case reported by Downs and Scott<sup>7</sup> and the third case of Soffer and Cohn.<sup>8</sup> It illustrates many of the changes that can occur in calcium and phosphorus metabolism, bones, kidneys and parathyroid glands as a result of disturbances of parathyroid and renal function.

### REPORT OF A CASE

A woman 43 years of age was first admitted to St. Barnabas Hospital July 6, 1940. She was single, working as an office clerk, and had been in fair health until 1933. Her illness apparently began in 1933 with progressive nervousness. She later became confused and was unable to think accurately or concentrate. She was forced to quit work. She complained of weakness and cardiac palpitation. At this time examination revealed nothing of note except a blood pressure of 148 systolic and 104 diastolic with a pulse rate of 106 per minute and evidence

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From the Minneapolis General Hospital, St. Barnabas Hospital and the Department of Pathology of the University of Minnesota Medical School.

1. Elsom, K. A.; Wood, F. C., and Ravdin, S.: Am. J. M. Sc. **191**:49, 1936.
2. Baker, B. M., Jr., and Howard, J. E.: Bull. Johns Hopkins Hosp. **59**: 251, 1936.
3. Bellin, D. E., and Gershwin, B. S.: Am. J. M. Sc. **190**:519, 1935.
4. Albright, F.; Baird, P. C.; Cope, O., and Bloomberg, E.: Am. J. M. Sc. **187**:49, 1934.
5. Curtis, L. E., and Feller, A. E.: Ann. Int. Med. **17**:1005, 1942.
6. Anderson, W. A. D.: Arch. Path. **27**:753, 1939.
7. Downs, R. S., and Scott, V.: Arch. Int. Med. **67**:658, 1941.
8. Soffer, L. J., and Cohn, C.: Arch. Int. Med. **71**:630, 1943.

of recent loss of weight. The urine showed a specific gravity of 1.016, a faint trace of albumin and nothing of significance on microscopic examination. She was discharged, August 21, with a diagnosis of psychoneurosis. Following this, her symptoms persisted in greater or lesser degree and were associated with numerous other vague complaints. In November of 1941 she began to complain of arthritic pain. She stated that all of her joints were stiff and painful. In May of 1942 she became sensitive to heat and cold. At about this time she noticed some deformity of the bones of her forearms and also increasing fatigue and weakness. She stated that her hats had become too small for her during the past year. She had experienced nocturia and diurnal frequency of urination for about one year. Recently her vision had been blurred, and she complained of a frequent dull headache. She reentered St. Barnabas Hospital on Aug. 18, 1942.

She was acutely ill and extremely emaciated. The frontal bones of the skull were prominent. The eyes, the ears, the nose and the throat were normal. The thyroid gland was slightly enlarged but was symmetric and of normal consistency. At the right lower pole was a small, barely palpable mass giving the impression of an adenoma of the lower pole of the thyroid gland. A definite systolic and diastolic bruit could be heard over the thyroid gland. The chest and the lungs were normal. The pulse rate was 120 per minute at rest, and the blood pressure was 140 systolic and 92 diastolic. The heart seemed slightly enlarged on percussion. The abdomen was pendulous, and the abdominal muscles lacked tonicity. The liver, the kidneys and the spleen could not be palpated. There was a noticeable bilateral bowing of the forearms. The long bones were sensitive to pressure. There was an atrophic type of dermatitis over both feet. The reflexes were equal and hyperactive. Laboratory examination revealed a hemoglobin content of 76 per cent (Sahli), with 9,650 leukocytes. A differential count showed 65 per cent neutrophils. The smear showed mild hypochromasia of the red blood cells. The urine had a specific gravity of 1.012, with a trace of albumin; microscopic examination revealed no abnormality. Bence Jones protein was absent. A Sulkowitch test for urinary calcium was positive with a 1 to 2 plus reaction. Serum calcium was 16.6 mg., and serum phosphorus was 6.8 mg., per hundred cubic centimeters; alkaline phosphatase was 52.3 King-Armstrong units. Total plasma protein was 5.5 Gm. per hundred cubic centimeters. Blood urea nitrogen was 51.8 mg. A recheck of the serum calcium showed a calcium level of 16.4 mg. and a phosphorus level of 6.15 mg. Roentgenologic examinations of the skull, the pelvis, the lumbar spine and the long bones were made. There was marked thickening of the bones of the skull, with areas of increased density and rarefaction. The changes in the skull suggested Paget's type of osteitis deformans. The pelvis, the left tibia, the right ulna and the radius, the left clavicle and the upper end of the left femur contained multiple cysts as found in generalized osteitis fibrosa cystica. August 22 a biopsy specimen was taken from a cystic lesion of the left clavicle. The biopsy showed osteitis fibrosa cystica. Two days later this clavicle fractured spontaneously. The clinical diagnosis was generalized osteitis fibrosa cystica due to a parathyroid adenoma.

August 25 the patient was operated on by one of us (C. O. R.).<sup>9</sup> The thyroid gland appeared entirely normal. Posterior to the right lower pole was a brownish red tumor measuring 3.5 by 2 by 1.5 cm. and weighing 3.17 Gm. (fig. 1). It was well encapsulated and extended into a recess in the posterior wall of the thyroid gland. The tumor was removed intact. Histologic examination showed

it to be a parathyroid adenoma composed largely of chief cells, with scattered areas of the vesicular cells present (fig. 2). Postoperatively the patient almost immediately presented signs of tetany. She became restless and nervous and later had several emeses. On the day after the operation the serum calcium dropped to 7.3 mg. per hundred cubic centimeters. The serum phosphorus remained elevated at 5.4 mg. Four days after the operation the serum calcium was 6.3 mg. The Sulkowitch test for urinary calcium became negative after the operation. The postoperative hypoparathyroidism was controlled by giving 1 cc. of parathyroid injection U. S. P. parenterally on two days. Following this, she received dihydrotachysterol. She was discharged from the hospital September 6 and was advised to take calcium, vitamin D and dihydrotachysterol. She felt greatly improved and had gained in weight and strength.\*

May 11, 1943 roentgenograms were made of the legs, the thighs, the clavicles, the skull and the right forearm. These showed an improvement in the cystic lesions involving the various bones. The lesions had not completely disappeared. For nearly two years after the operation the patient felt well. She continued to take calcium by mouth and vitamin D. In her third postoperative year she complained of nocturia and excessive thirst. She later became weak and suffered from severe nausea and vomiting. She was admitted to the Minneapolis General Hospital on Aug. 22, 1945. Physical examination revealed an apathetic, emaciated woman 48 years of age. Her blood pressure was 90 systolic and 70 diastolic. The skin had a dark color and was very dry. The temperature, the respirations and the pulse were normal. There were scattered areas of ecchymosis measuring up to 2 cm. in diameter over the trunk and the extremities. The heart and the lungs revealed no abnormalities. The abdominal regions appeared normal. The extremities were extremely tender on pressure. Chvostek's sign was questionably positive. The patient was somewhat confused and disoriented and complained of multiple pains. The hemoglobin content was 72 per cent (Sahli). Serologic tests revealed no syphilis. The leukocyte count was 10,400, with 81 per cent neutrophils. The urine had a specific gravity of 1.015, contained albumin (3 plus) but no sugar and showed one to two red blood cells per high power microscopic field. Blood sugar was 128 mg. per hundred cubic centimeters; the carbon dioxide-combining power was 47 volumes per cent; the serum calcium was 8.77 mg. per hundred cubic centimeters and when rechecked was found to be 6.4 mg. Serum phosphorus was 10.2 mg. Alkaline phosphatase was 10.4 King-Armstrong units. Serum albumin was 6.4 Gm. and serum globulin was 3.0 Gm. per hundred cubic centimeters. Blood urea nitrogen was 174 mg., with 6.3 mg. of creatinine. Roentgenograms of the skull and long bones showed changes that were consistent with partially healed generalized osteitis fibrosa cystica. In some areas the process appeared to be still active suggesting persistent hyperparathyroidism. Punched-out areas were present in the skull. Aspirated sternal marrow revealed no evidence of any blood dyscrasia, multiple myeloma or metastatic carcinoma. A second determination of carbon dioxide-combining power showed it to be 19 volumes per cent. The clinical diagnosis was uremia. She died on Aug. 28, 1945.

*Postmortem Examination.*—The body was that of a fairly well developed but poorly nourished white woman of middle age. There was no jaundice or edema. There were scattered areas of ecchymosis over the surfaces of the legs and the arms. The peritoneal cavity contained about 200 cc. of amber fluid. The surfaces were smooth and glistening. The right pleural cavity contained about 500 cc. of straw-colored fluid and the left 300 cc. of similar fluid. The pericardial sac appeared normal. The heart weighed 250 Gm. and appeared normal. The right

lung weighed 260 Gm and the left 410 Gm. Both lungs showed slight edema and small areas of hypostatic pneumonia. The liver weighed 1,320 Gm and was not remarkable. The bile ducts were patent, and the gallbladder appeared normal. The spleen weighed 100 Gm. The gastrointestinal tract appeared normal. The right kidney weighed 90 Gm. and the left 110 Gm. The capsules stripped with ease from surfaces that were granular and deeply scarred. The right kidney showed considerable atrophy of the parenchyma, as the cortex and medulla in

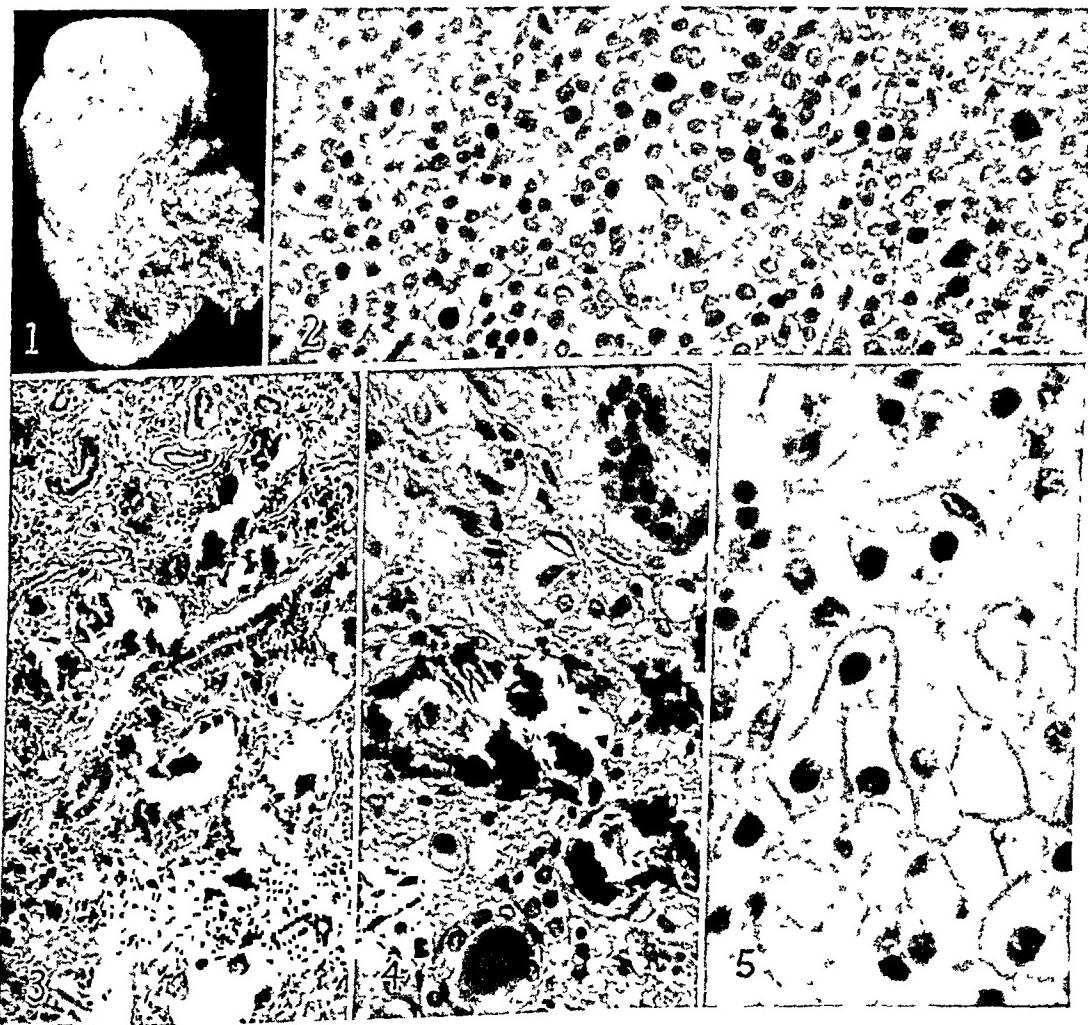


Fig. 1.—Parathyroid adenoma.

Fig. 2—Photomicrograph of the parathyroid adenoma.

Fig. 3—Photomicrograph of kidney showing cystic spaces filled with calcium

Fig. 4—Photomicrograph of kidney showing calcium deposited in tubules.

Fig. 5—Photomicrograph of parathyroid gland at autopsy showing preponderance of "wasserhelle" cells.

some areas measured only 3 mm in thickness. The cortex of the left kidney measured 3 mm. and the medulla 10 mm at the widest point. The markings between the cortex and the medulla were indistinct in both kidneys. The right kidney contained, in addition, a number of small cortical cysts measuring up to

2 cm. in diameter. Stones or gross evidence of calcification were not seen in either kidney. The pelvis of the right kidney showed slight dilatation. The left pelvis appeared normal. The ureters and the bladder appeared normal. The genital organs revealed nothing of note. The aorta showed moderate sclerosis. There was no enlargement of the thyroid gland. Two parathyroid glands were found in the connective tissue behind the left lobe of the thyroid. The remaining parathyroid gland of the right side could not be found. The two parathyroid glands found measured 5 by 3 by 2 mm. each. The ribs and the sternum showed no gross abnormalities. The skull, the long bones, the brain and the spinal cord were not examined.

*Microscopic Examination.*—Kidneys: Under low magnification the most impressive alteration was the presence of calcium deposits throughout the parenchyma (fig. 3). The calcium was much more abundant in the medullary pyramids than in the cortex. In the pyramids there were many cystic spaces filled with calcium, which were almost of macroscopic dimensions. There was also rather marked patchy atrophy of the cortical tubules, and some of the intact tubules were moderately dilated. Under high magnification it appeared that the cystic spaces in the pyramids represented collecting tubules distended with calcium. Since the lining epithelial cells had disappeared at the site of the calcium deposit, it was not easy to determine whether the calcium was intratubular or interstitial. Nearby all the collecting tubules were destroyed. A careful study revealed that the calcium in the cortex was intratubular (fig. 4) although the destruction of the tubular epithelium often caused it to appear as a deposit in the interstitial tissue. There was a moderate lymphocytic exudate throughout the atrophic areas. In addition to the obstructive tubular lesion, which was the cause of the uremia, there were a number of hyaline glomeruli associated with atrophic tubules. Some of the hyaline glomeruli were surrounded by old fibrous crescents. The vast majority of the glomeruli were normal.

Parathyroid Glands: Sections of the two remaining glands found at autopsy showed a marked preponderance of large clear *wasserhelle* cells, with a few oxyphil and chief cells present (fig. 5).

Remaining Organs: The sections showed nothing of note other than an inflammatory exudate in the lungs as found in the terminal stage of bronchopneumonia.

#### COMMENT

The patient's condition can be classified as primary hyperparathyroidism due to an adenoma of one parathyroid gland. The symptoms related to the skeletal system began about nine years before operation. There was no history of any preceding renal disease. The changes in the blood chemistry were the result of hyperparathyroidism as modified by secondary renal insufficiency. The serum calcium levels of 16.6 mg. and 16.4 mg. were obtained before operation at the time the serum phosphorus was 6.8 mg. This did not fit the usual picture of hyperparathyroidism, as one expects a reciprocal relationship of low phosphorus with high serum calcium. The answer was found in the blood urea nitrogen level of 52.8 mg. This indicates that at the time of the operation there was a considerable degree of renal insufficiency with loss of the ability to excrete phosphates. The serum calcium, however, remained high under the influence of the parathyroid adenoma. Following operation, the serum calcium fell to the tetanic levels of 7.3 mg. and 6.3 mg. This is positive evidence that the hyperparathyroidism was due to the adenoma and not to diffuse hyperplasia of all four

glands. The serum phosphorus remained elevated at 5.4 mg. This elevation of serum phosphorus may have been another factor in the depression of the calcium level after the stimulus of the parathyroid adenoma had been removed. Unfortunately, further studies of both calcium and phosphorus metabolism were not done until the patient's final admission to the Minneapolis General Hospital three years later. At this time her serum calcium varied between 6.4 mg. and 8.7 mg., while the serum phosphorus was 10.2 mg. The histologic discovery of the *wasserhelle* cells in the remaining parathyroid glands suggests a compensatory hyperfunctioning state to compensate for the depression of serum calcium levels. The findings in the long bones and the skull shortly before death suggested both a healing of the initial lesions associated with osteitis fibrosa cystica and further progression of the disease within the bones. The serum phosphorus rose from 5.4 mg. to 102 mg. during this three year postoperative period. The blood urea nitrogen rose from 51.8 mg. to 174 mg. during the same period. The reciprocal relationship between phosphorus and calcium levels placed a severe strain on the calcium metabolism postoperatively and led to a state of secondary hyperparathyroidism.

The renal destruction as a result of the deposition of calcium was extensive. The tubular blocking appeared to be of long standing and to have arisen in the years prior to her operation. Dr. E. T. Bell studied the kidneys in this case for us. The old fibrous crescents about the hyaline glomeruli indicated that the patient at one time had glomerulonephritis and that a number of glomeruli had been destroyed. The hyalinization of a small percentage of the glomeruli was not the cause of the renal insufficiency. This was clearly due to the widespread presence of calcium casts obstructing the tubules, notably the collecting tubules. The advisability of long-continued postoperative oral administration of calcium and vitamin D has to be questioned because of the danger of further calcification of the kidneys, already damaged. Tumulty and Howard<sup>10</sup> showed that excessive intake of calcium with massive vitamin D therapy may cause prolonged hypercalcemia and persistent impairment of renal function in normal persons. The fact that this patient responded well to the surgical removal of the adenoma, with a state of fair health resulting over a period of two years, would justify the removal of a parathyroid adenoma even in the presence of considerable renal damage. The prognosis for complete cure seems poor.

The parathyroid adenoma consisted largely of chief cells, with scattered areas of large vesicular cells. The *wasserhelle* cells were absent. On the other hand, the parathyroid glands removed at autopsy were composed almost entirely of *wasserhelle* cells. The latter picture has been described by Castleman and Mallory<sup>11</sup> as typical of diffuse primary hyperplasia, but in this case all evidence points to the change being secondary to the renal insufficiency. Nelson<sup>12</sup> found no constant uniformity in the histologic aspects of the parathyroid glands in cases

10. Tumulty, P. A., and Howard, J. E.: J. A. M. A. **119**:233, 1942.

11. Castleman, B., and Mallory, T. B.: Am. J. Path. **13**:553, 1937.

12. Nelson, A. A.: Arch. Path. **24**:30, 1937.

of renal insufficiency, as in some cases reported the glands were composed mainly of chief cells; in others, of *wasserhelle* cells, and in others, of various mixtures and transitional forms.

#### SUMMARY

Primary hyperparathyroidism due to a parathyroid adenoma caused uremia in a 45 year old woman. Death occurred three years after the surgical removal of the parathyroidoma. Renal insufficiency was present at the time of the operation, with subsequent elevations of the blood levels of phosphorus and urea nitrogen. At autopsy uremia was found to be due to calcification of the tubules of the kidneys. The roentgenograms of the bones and the appearance of the remaining parathyroid glands when encountered at autopsy suggested a postoperative state of gradually increasing secondary hyperparathyroidism due to the progressive renal failure.

## SPLENIC HEMANGIOSARCOMA A Case with Lymphatic and Vascular Metastases

DONALD DE F. BAUER, M.D., and W. RANEY STANFORD, M.D., DURHAM, N. C.

**A**T LEAST 9 cases of primary splenic hemangiosarcoma have been reported, as outlined in tables 1 and 2. This rare tumor has usually been recognized on microscopic examination after splenectomy or autopsy. In most of the reported cases the spleen was three to ten times normal size. Symptoms (and sometimes death) were related to splenomegaly and hemorrhage from the primary site. With the exception of several cases in which curative splenectomy was accomplished before metastases occurred, secondary growths in the liver were common. Metastases elsewhere have not been commonly noted, and metastases in lymph nodes have not been previously reported.

### REPORT OF CASE

A 32 year old white married woman bore a full term, healthy child in the summer of 1942. For relief of repeated sore throats, tonsillectomy had been performed in the seventh month of her pregnancy. About that time she complained of weakness, which persisted until her death. In May 1943 pain began in the right side of the chest, accentuated by deep respiration. Following delivery, her sore throats had recurred; therefore in June 1943 some tonsillar tags were removed. In July she complained of a sensation of fulness in the upper part of the abdomen and shortness of breath on exertion. A nonproductive cough was noted. She was afebrile and was not losing weight. In August she entered the hospital, complaining of "pleurisy." Enlargement of the posterior cervical lymph nodes was noted. The node at the angle of the right mandible was large, fixed and not tender. During her stay in the hospital it was excised and examined (a report is given later in this article). Bilateral pleuritic pain was experienced on deep inspiration; expansion was poor, and voice and breath sounds were slightly altered. Tenderness was noted at the right costal margin. The hemoglobin content was 84 per cent; the erythrocyte count, 4,500,000, the leukocyte count, 9,600. The urine was normal. The basal metabolic rate was minus 7. The sputum when examined showed nothing remarkable. Dextrose tolerance tests gave results within normal limits. The sedimentation rate was persistently elevated. A roentgenogram of the chest showed soft, small, mottled shadows in the lower lung fields, increased linear markings and increased density about the right hilus. Roentgenograms taken at intervals of several weeks showed continued increase in the density of the linear markings. A lateral roentgenogram of the skull showed one clear, fairly smooth, rounded area of decreased density in the right occipital region, probably in the outer table. In the roentgenograms of the spine, areas of decreased density were seen in the second and third lumbar bodies.

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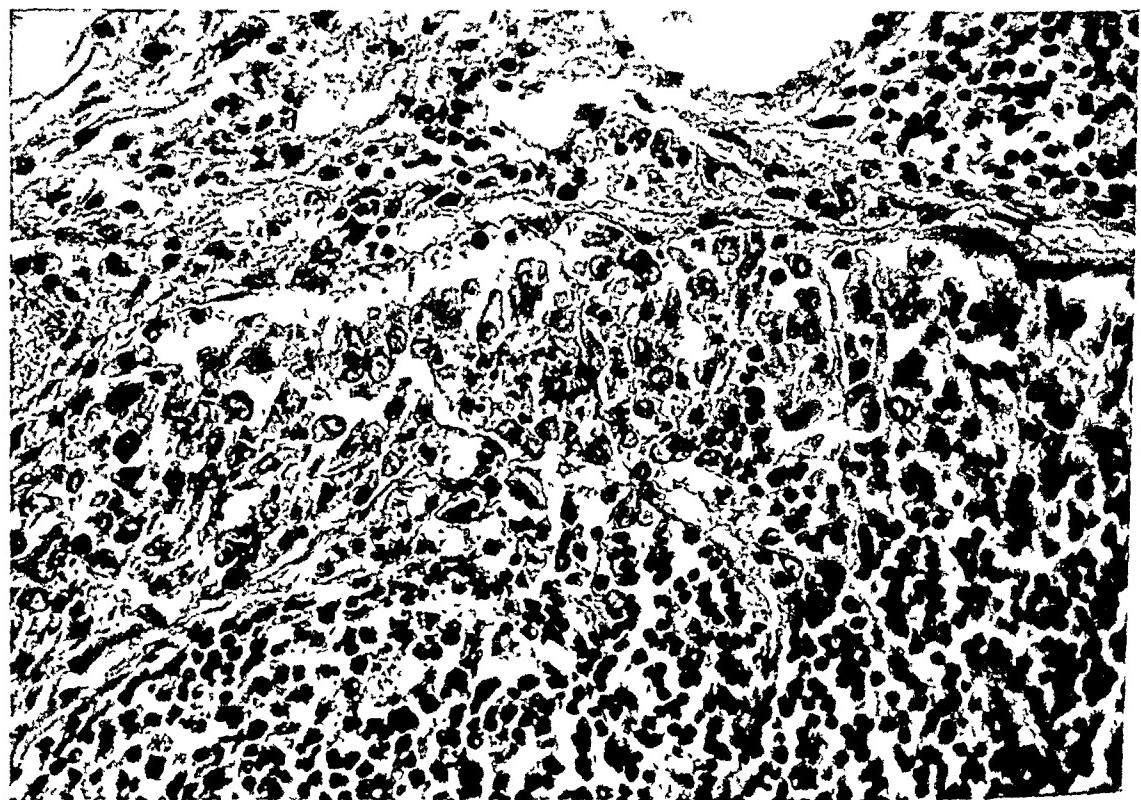
From the Department of Pathology, Duke University School of Medicine and Duke Hospital, and the Department of Medicine, Watts Hospital.

The patient complained often of pain in the lumbar region and the lower part of the right hemithorax. Her throat was "sore" much of the time, but nothing to explain this was found. Biopsy of the right submandibular node indicated the probability of a cancer metastasizing to the cervical lymph nodes, primary site unknown. The recognition of an "atypical growth of polyhedral cells with large nuclei and indefinite cell outlines, the cells looking as though of epithelial origin," and the finding of infrequent mitotic figures, left small room for question whether the illness was infectious or neoplastic. The patient left the hospital in September 1943.

Her course during the month's interval before the terminal admission was characterized by a gradual onset of hemoptysis, increased weakness and slight pyrexia. On admission she was acutely ill, very orthopneic, dyspneic and cyanotic. Her temperature was 39.8 C. (103.6 F.); the pulse rate, 140; the blood pressure, 100 systolic and 80 diastolic. She was still adequately nourished. Submandibular and submaxillary nodes were slightly enlarged and not tender. The chest was dull to percussion in the axillas and the bases of the lungs. Coarse, sticky rales were heard. The abdomen was distended, with the liver palpable 3 fingerbreadths below the costal margin. The hemoglobin content was 75 per cent; the erythrocyte count, 3,900,000; the leukocyte count, 17,900. The patient became rapidly worse despite oxygen therapy, dying sixteen hours after admission, one and one-half years after the onset of the illness.

*Autopsy.*—The spleen was average size (145 Gm.). Externally there was nothing to suggest the presence of the spherical tumor 2.5 cm. in diameter discovered on section. The remainder of the spleen was entirely normal in consistency, color and structure except for a few light yellow tubercles immediately beneath the capsule. The tumor was a single circumscribed round nodule, bulging from the cut surface. It was mottled purple and dark gray, and therefore presented little contrast to the surrounding normal splenic tissue. Several pancreatico-lienal, hepatic and periaortic lymph nodes were slightly enlarged. On section these were mottled with gray-purple areas. The 1,570 Gm. liver had many capsular-diaphragmatic adhesions. In such places there were gumma-like lesions with gray-purple color, necrotic-looking centers and multiple pseudopod-like peripheral extensions into the hepatic parenchyma. Most of these were 1 to 3 cm. in diameter. Within the depths of the organ were similar areas, some of which were strikingly hemorrhagic. The right pleural cavity contained 2,000 cc. of sanguineous fluid without gross blood clot. A moderate quantity of fibrin was found on the right pleural surfaces, and one basilar adhesion. There was neither adhesion nor effusion on the left. A marked contrast was noted bilaterally between the purple color of most of the visceral pleural surface and the white color of the broad linear trabecular markings. The lungs were noncrepitant and extraordinarily firm, as though completely solidified. A decidedly hemorrhagic tissue was revealed by section, but diffusely scattered areas of air-containing lung substance persisted. Together the lungs weighed 1,560 Gm. The pigmented hilar nodes were not enlarged. Hemorrhagic, spongy areas were encountered in the bodies of the twelfth thoracic and second and third lumbar vertebrae. These averaged 2 cm. in diameter. The 250 Gm. heart was dilated in all chambers. The right side was more dilated than the left. Permission was refused for examination of the brain and the area of decreased radiodensity in the skull which was reported in the clinical studies.

*Microscopic Examination.*—Spleen: Sections from outside the grossly visible tumor area showed no lesions except several fibrocaseous tubercles immediately



Upper: The characteristic reticulum of the tumor (lower part) is shown in contrast with the normal reticulum of the adjacent normal splenic tissue ( $\times 110$ ).

Lower: Microscopic appearance of a solid tumor metastasis in the peripheral sinus of a pancreaticoileal lymph node ( $\times 341$ ).

beneath the capsule. The circumscribed area of tumor was not encapsulated. The transition between normal splenic and neoplastic tissue was gradual, being evidenced most obviously by increased vascularity. There was, however, an abrupt change in the reticulum, as revealed by the Perdrau stain. In the tumor the reticulum was denser. Mallory's connective tissue stain left no doubt that there was an increase in collagenous tissue. The principal cellular component of the tumor was a pleomorphic cell. The cells lined the irregular blood-filled channels, often forming a stratified layer two or three cells thick. Tumor-containing thrombi and tumor cells without thrombi were found within normal splenic blood vessels. In some places the tumor cells simulated fibroblasts; elsewhere, macrophages; in still other places, endothelial cells lining the normal splenic sinuses. They were poor in cytoplasm, but protoplasmic processes, apparently connecting with the reticulum, were discernible in places. The clear ovoid nuclei contained granules and threads of basophilic material, principally at the periphery. They varied between 10 and 20 microns. Nucleoli were not found. Mitoses and multi-nucleated cells were rare. Zones of necrosis were not found.

**Tumor in Other Sites:** The features described for the splenic nodule were characteristic of the metastases found in abdominal lymph nodes, the liver, the lungs, the mediastinal lymph nodes and the vertebrae. Necrotic changes were most pronounced in the liver. Because there was a moderate grade of central necrosis secondary to passive congestion outside the tumor areas in the liver, it seems improper to ascribe much weight to the degree of necrosis within the hepatic tumor in comparing the neoplastic lesions in the spleen and the liver as to age. The tumor growth in the pleura, the pleural lymphatics and the interlobular septums was extensive. Hemorrhage was widespread, completely filling many alveoli and bronchioles. The tumor stroma was densest in the vertebral site, where relatively avascular hyalinized regions were observed.

**Other Tissues:** Sections of the thymus, the submaxillary gland, the thyroid gland, the breast, the pancreas, the adrenal glands, the kidneys, the ileum, the heart and the ribs showed no tumor. No trace of tonsillar tissue remained for microscopic study.

#### COMMENT

Two tables are included in this report for the purpose of demonstrating the similarities and the differences between this and the previously reported cases of cancerous primary splenic hemangioma.

The extraordinary papillary projections noted by Wright<sup>1</sup> were not present in this case. Apparently the abnormal vascular channels elaborated by hemangiosarcoma are connected with the normal blood vessels and function, however inadequately, as a blood supply for the tumor cells which line them. In cases in which necrosis of tumor was noted in the spleen, thrombi and infarcts were prominent.

The small size of the tumor in the spleen observed by us might be considered as an argument against the conclusion that the spleen was the primary site. The following were considered as possible sites of the primary tumor: (1) tonsils; (2) bones, especially the vertebral bodies; (3) liver; (4) spleen. The conclusion that the spleen was the primary site is based on the character of the tumor itself and the general character and distribution of the metastases. In the spleen

1. Wright, A. W.: Am. J. Path. 4:507, 1928.

the tumor was a single circumscribed nodule. Everywhere else it was multiple and infiltrating. The characteristic microscopic structure was nowhere as definitive as in the spleen. It may be significant that intravascular growth was venous in the spleen and primarily portal in the liver. Lymphatic spread from the spleen was suggested by the involvement of the pancreaticocolic and hepatic lymph nodes. In most reported cases the spleen was known to be involved in some sort of

TABLE 1.—*Gross Features of Reported Cases*

Author	Date	Wt. of Spleen, Gm.		Age	Race	Sex	Metastases
		525	57				
DeNavasquez, S.: J. Path. & Bact. 42: 651, 1936	1936	525	57	White	M	Liver	
Garlock, J. H.: J. Mt. Sinai Hosp. 6: 319, 1940	1940	635	13	White	F	Splenectomy; recovery	
Homans, J.: Ann. Surg. 25: 732, 1897	1897	415	22	White	F	No autopsy	
Jopson: S. Clin. North America 2: 235, 1921	1921	1,590	33	White	F	Splenectomy; recovery	
Von Jores, L.: Zentralbl. f. allg. Path. u. Path. Anat. 19: 662, 1908	1908	3,600	45	White	F	Liver	
Langhans, T.: Virchows Arch. f. path. Anat. 75: 273, 1879	1879	2,880	30	White	M	Liver	
Paine, C. G.: J. Path. & Bact. 34: 139, 1931	1931	1,300	61	White	M	Liver and bone	
Theile: Virchows Arch. f. path. Anat. 1904	1904	2,500	56	White	M	Liver, lungs and stomach	
Wright <sup>1</sup> .....	1928	520	25	White	M	Liver	
Bauer, D. deF., and Stanford, W. R.: Arch. Path., this issue	1944	145	32	White	F	Liver, pleura, lungs, bronchial, cervical and retroperitoneal lymph nodes and vertebrae	

TABLE 2.—*Microscopic Features of Reported Cases*

Author *	Cell Type	Giant Cells	Mitoses	Solid Growth	Degenerative Changes
DeNavasquez.....	Spindle	Present	Numerous	Present	Liver only
Homans.....	.....	.....	.....	.....	Hyalinization
Jopson.....	Spindle	Present	.....	Present	Hyalinization
Jores.....	.....	.....	.....	.....	Marked necrosis
Langhans.....	Fusiform	.....	.....	.....	.....
Paine.....	Spindle to circular	Present	Rare	Present	Necrosis
Theile.....	Endothelial	.....	.....	Present	.....
Wright.....	Large vesicular	.....	Numerous	Present	Necrosis
Bauer and Stanford	Polyhedral	Present	Rare	Present	Necrosis

\* For complete references, see table 1.

disease process because of its large size. Here the small size of the primary splenic tumor removed the possibility of diagnosing the ailment from localizing symptoms.

An additional factor which increased the difficulty of diagnosis was the surprisingly extensive growth of the tumor in the lungs. Although it soon became apparent that the process was neoplastic rather than infectious, interest continued to center in the progressive pulmonary

involvement because the most disturbing of the symptoms and the cause of death could be traced to this. Increasing obstruction of pulmonary vessels by embolism and the pressure of tumor growth resulted in progressively rising pulmonary blood pressure. The right ventricle was at last incapable of further compensation. The massive right pleural effusion was one evidence of the decompensation which ensued. Concomitant atelectasis from pressure of this effusion on the small amount of remaining lung tissue of the right side further increased the cardiac burden. When the load was then no longer bearable, cardiac dilatation occurred with general circulatory failure and death.

#### SUMMARY

Of the 7 reported cases of splenic hemangiosarcoma in which autopsy was performed, the case presented here is the only one in which metastases were demonstrated in lymphatic and lymphoid structures. This case is likewise interesting because of the small size of the primary tumor in relation to the large size and the wide distribution of the metastases. The wide distribution of intrapulmonary metastases produced pulmonary hypertension and myocardial failure.

# Laboratory Methods and Technical Notes

## A RAPID GRAM STAIN FOR TISSUE

EDWIN M. LERNER II, M.D., BOSTON

**A** NEW modification of the Gram stain for the demonstration of bacteria in tissues has been employed in this laboratory with success. The principal objections to the usual modifications of the Gram stain previously employed are: the length of time required, the numerous and sometimes complicated steps in the procedures and the fact that many of the Gram stains on tissue give little or no differentiation between gram-positive and gram-negative organisms. Furthermore, the commonly employed Gram-Weigert modification gives poor delineation of both bacterial and tissue detail.

### PROCEDURE

The procedure is essentially that of the Gram stain commonly employed for bacterial smears. In point of fact, the staining may be done on individual slides in a fashion identical with that used for staining bacterial smears. It is more convenient, however, when staining several slides at once to use staining jars.

### PREPARATION OF SLIDES

Fix tissue in Zenker's solution twenty-four hours.

Embed in paraffin.

Cut sections 6 microns thick.

Mount on slides and heat at 56 C. two to six hours.

### STAINING SOLUTIONS

#### *Stirling's Gentian Violet Solution*

Crystal violet.....	5 Gm.	Iodine .....	1 Gm.
Absolute alcohol.....	10 cc.	Potassium iodide.....	2 Gm.
Aniline .....	2 cc.	Distilled water.....	100 cc.
Distilled water.....	88 cc.		

#### *Iodine Solution*

#### *Decolorizing Agent*

Acetone .....	50 cc.	1 per cent aqueous solution
Absolute alcohol.....	50 cc.	

#### *Safranin*

From the Department of Pathology, Children's Hospital and Infants' Hospital.

## METHOD OF STAINING

1. Treat paraffin sections in xylene ten minutes, then in absolute alcohol ten minutes; rinse in 95 per cent alcohol.
2. Stain with Stirling's gentian violet one minute.
3. Wash in water.
4. Treat with iodine solution one minute.
5. Wash in water.
6. Decolorize with alcohol-acetone mixture until no more stain comes away (ten to thirty seconds).
7. Wash in water.
8. Counterstain with safranin thirty seconds.
9. Wash in water.
10. Rinse in 95 per cent alcohol until excess safranin is removed.
11. Rinse in absolute alcohol, then in xylene, and mount in balsam.

## RESULTS

Gram-positive organisms are stained deep blue to violet; gram-negative organisms, red. The nuclei of cells are stained pink to red, with excellent intranuclear detail, and the cytoplasm is stained light yellowish pink. Bacterial spores are easily visible as clear spaces within the cells.

## COMMENT

The rapid Gram stain for differential staining of bacteria in tissues requires less than five minutes of actual staining time for its execution and has the added advantage of being extremely simple. It gives excellent morphologic detail of both bacteria and tissues, although the latter are stained somewhat lightly. Spore-bearing bacteria can be recognized readily. Bacteria are sharply delineated whether occurring in numbers or scattered individually through the tissues. The finding of occasional scattered organisms is thus facilitated.

## A HEMOGLOBIN STAIN FOR HISTOLOGIC USE BASED ON THE CYANOL-HEMOGLOBIN REACTION

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BETHESDA, MD.\*

THE USE of cyanol as a stain for hemoglobin was suggested by Fautrez and Lambert,<sup>1</sup> whose technic required one to twenty-four hours. In addition, the preparation of the staining solutions called for precisely made buffers and boiling of carmalum for one hour. This elaborate preparation was undoubtedly necessary in staining the delicate hemoglobin globules in the renal epithelium of Urodeles, as they did, but with larger laboratory animals much simpler technics usually suffice for the identification of hemoglobin.

Fautrez and Lambert did not believe cyanol was acting as a peroxidase stain in their hemoglobin-staining method. However, cyanol, color index no. 715, is closely related chemically to patent blue V, color index no. 712, and this chemical relationship suggested that it might be used in Lison's technic<sup>2</sup> as modified by Dunn.<sup>3</sup>

By thus substituting cyanol for patent blue V, a very satisfactory hemoglobin stain is obtained. This cyanol-peroxidase reaction has been found to be highly specific for hemoglobin (erythrocytes, hemoglobin-containing renal casts and globules) in paraffin sections of tissue fixed in 4 per cent solution of formaldehyde buffered to  $p_H$  7.0. It has been tested on renal tissue of hemoglobinuric mice, rats, guinea pigs, dogs and monkeys. Hemoglobin stains dark blue to light bluish gray. The specificity and sensitivity of this cyanol hemoglobin stain is equal to that of patent blue V, in most instances. Cyanol is also useful on frozen sections and methylalcohol-fixed blood smears, but it is not recommended for the latter purpose unless the smear is very well fixed, as the blood film often becomes detached.

### \*TECHNIC

The cyanol stock solution is prepared by dissolving 1 Gm. of cyanol<sup>4</sup> in 100 cc. of distilled water, then adding 10 Gm. of zinc powder, chemically pure and 2 cc. of glacial acetic acid. This mixture is brought to the boiling point. In a short time complete decolorization (loss of blue color) will occur. This solution is stable for several weeks. To prepare working reagent: Filter, just before use, 10 cc. of stock solution and add 2 cc. of glacial acetic acid and 1 cc. of commercial hydrogen peroxide (3 per cent).

\* From the Pathology Laboratory, National Institute of Health.

1. Fautrez, J., and Lambert, P. P.: Bull. d'histol. appliq. à la physiol. **14**: 29, 1937.

2. Lison, L.: Beitr. z. path. Anat. u. z. allg. Path. **101**:94, 1938.

3. Dunn, R. C.: A Simplified Stain for Hemoglobin Using Patent Blue, to be published.

4. The cyanol (color index 715) used was furnished by National Aniline Division, Allied Chemical & Dye Corporation.

To prepare safranin counterstain: Make a 1:1,000 safranin solution in 1 per cent acetic acid.

To stain sections: Bring sections to water; stain in freshly prepared working reagent three to five minutes; rinse in water; counterstain in the safranin solution about one minute; rinse in water; dehydrate with alcohols; clear in xylene; mount in clarite.

Results: Hemoglobin stains dark blue to bluish gray, the nuclei red and the cytoplasm light pink.

The total time required for staining is about ten minutes.

#### SUMMARY

The hemoglobin stain described may be used to identify hemoglobin in frozen or paraffin sections of tissue fixed in 4 per cent solution of formaldehyde buffered to  $p_H$  7.0.

The deparaffinized, hydrated sections are stained in the cyanol working reagent about four minutes, washed, counterstained in acidified safranin about one minute, rinsed in water, dehydrated and mounted in clarite. The time required exclusive of hydration and dehydration is about ten minutes. Hemoglobin stains blue to bluish gray.

## Notes and News

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**Appointments, Etc.**—C. G. Tedeschi, Medfield State Hospital, Harding, Mass., has been appointed director of the laboratory at Framingham Union Hospital, Framingham, Mass.

Birmingham Medical College, Birmingham, Ala., has announced appointments as follows: in pathology, R. D. Baker professor, J. D. Bush associate professor, A. E. Casey and J. A. Cunningham assistant professors; in bacteriology and clinical pathology, R. McBurney professor and W. H. Riser associate professor of clinical pathology.

William C. White, Washington, D. C., has resigned as chairman of the committee on medical research of the National Tuberculosis Association, ending a service of more than twenty-five years. Under his chairmanship the committee made about fifty grants in support of research, amounting in all to some \$600,000.

S. W. Lippincott, recently released from the Army Institute of Pathology, has been appointed professor of pathology and executive of the department of pathology at the new school of medicine of the University of Washington.

**Death.**—Simon Flexner, pathologist and bacteriologist, the first director of the Rockefeller Institute of Medical Research, died May 2, 83 years old.

**Award.**—O. T. Avery, of the Rockefeller Institute for Medical Research, New York, has been awarded the Kober Medal of Georgetown University School of Medicine, Washington, D. C., for his work on problems of pneumonia.

**Committee on Growth of the National Research Council.**—This committee, which acts for the American Cancer Society, will consider applications for grants in aid of cancer research to become effective July 1, 1947. Applications will be received until Sept. 15, 1946. Applications for fellowships in cancer research for the ensuing year will be received until Dec. 1, 1946. Application for grants for the current year will no longer be entertained.

To date the committee has recommended seventy-five research grants and fourteen fellowships. It will continue to recommend support of research in the basic sciences and in clinical investigative medicine broadly pertaining to problems of growth. The address is Committee on Growth, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C.

**Endowment for Surgical Research.**—Theodore L. Case, retired Philadelphia surgeon, has given Temple University School of Medicine \$1,000,000 to establish a surgical research foundation with special emphasis on cancer.

# INDEX TO VOLUME 41

Subject entries are made for all articles. Author entries are made for original articles. Book Reviews and Obituaries are indexed under these headings in alphabetical order under the letters B and O, respectively.

- Abdomen:** See Gastrointestinal Tract; etc.  
**Abnormalities and Deformities:** See under names of organs and regions, as Spine; etc.  
**Abscess:** See under names of organs and regions, as Liver; etc.  
**Acetylcholine:** See Choline and Choline Derivatives  
**Acid, Amino:** See Amino Acids  
**Actinomycosis:** reticulum fibers in, 310  
**Adenoacanthoma of stomach:** report of case, 213  
**Adenocarcinoma of urachus involving urinary bladder:** 388  
**Adenohypophysis:** See Pituitary Body  
**Adenoma:** parathyroid adenoma with uremia due to calcification of kidneys, 661  
reticulum fibers in, 315  
**Adipose Tissue:** See Fat; Lipoma; Obesity  
**Adrenal Preparations:** microscopic lesions in acetylcholine shock, 11  
**Adrenals, hemorrhage in meningococcic sepsis:** 503  
**Agglutinins and Agglutination:** See Blood, groups; etc.  
**Ainhum:** etiologic concepts and pathologic aspects of, 639  
**Alloxan:** hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
**Altshul, R.:** Microscopic lesions in acetylcholine shock, 11  
**Amebiasis:** amebic dysentery, 107  
**Amino Acids:** morphologic studies of rats deprived of essential amino acids; histidine, 25  
**Amyloid, improved methods for demonstrating in paraffin sections:** 559  
**Anaphylaxis and Allergy:** See also Shwartzman Phenomenon; etc.  
microscopic lesions in acetylcholine shock, 11  
transitory pulmonary infiltrations (Loeffler's syndrome) in rabbits, 489  
**Ancylostomiasis:** See Hookworm Infection  
**Anemia, hemolytic:** acute febrile illness characterized by thrombopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327  
**Angioma:** reticulum fibers in tumors of blood and lymph vessels, 313  
**Animals:** See under Dogs; etc.  
**Anoxemia:** See Blood, oxygen  
**Antigens and Antibodies:** See Blood, groups; and under specific antigens and reactions  
**Antiserum:** See under names of various diseases  
**Aorta, genesis of aortic perforation secondary to carcinoma of esophagus:** report of observations in 2 cases, 533  
obstruction of aortic Isthmus by calcified thrombus, 63  
**Apparatus, portable exhibit case:** 556  
**Appointments:** 120, 220, 341, 454, 563, 678  
**Armies:** See Military Medicine  
**Arteries:** See also Aorta; Arteriosclerosis; Blood pressure; Embolism; Thrombosis; etc.  
**Coronary:** See Coronary Vessels  
**Hypertension:** See Blood pressure, high
- Arteries—Continued**  
inflammation; hypertension and necrotizing arteritis in rat following renal infarction, 231  
local tissue reactivity (Shwartzman phenomenon) in heart and femoral artery of rabbit, 565  
**Renal:** See under Kidneys
- Arteriosclerosis:** experimental studies in cardiovascular pathology; atheromatosis in dogs following repeated intravenous injections of solutions of hydroxyethylcellulose, 130  
experimental studies in cardiovascular pathology; vibratory lability of plasma colloids in rabbits and in dogs following ingestion of cholesterol, 139
- Arteritis:** See Arteries, inflammation
- Arthritis:** See Gout; Rheumatism
- Ascariasis:** 109
- Ashworth, C. T.:** Renal lesions in portal cirrhosis, 476
- Atheromatosis:** See Arteriosclerosis
- Atherosclerosis:** See Arteriosclerosis
- Atrophy:** See under names of organs and regions
- Autography:** See Goiter
- Awards:** 120, 220, 341, 563, 678
- Axilrod, H. D.:** Obstruction of aortic isthmus by calcified thrombus, 63
- Bacilli:** See Bacteria
- Bacteria:** 78. See also under Meningococci; Streptococci; Viruses; etc.  
**Calmette-Guérin:** See Tuberculosis
- Dysentery:** See Dysentery
- Enteritidis:** See Salmonella enteritidis
- Leprosy:** See Leprosy
- Paratyphoid:** See Salmonella
- Pestis:** See under Plague
- rapid gram stain for tissue, 674
- Shigella:** See Dysentery
- Baker, A. B.:** Tropical diseases; involvement of nervous system, 66
- Bartonellosis:** See Oroya Fever
- Basedow's Disease:** See Goiter, exophthalmic
- Bauer, D. deF.:** Splenic hemangioscarcoma; case with lymphatic and vascular metastases, 668
- Baxter, J. H.:** Renal lesions in portal cirrhosis, 476
- Becks, H.:** Changes in central incisors of hypophysectomized female rats, after different postoperative periods, 457
- Bell, E. T.:** Relation of cholelithiasis to acute hemorrhagic pancreatitis, 17
- Besnier-Boeck's Disease:** See Sarcoidosis
- Bilharziiasis:** See Schistosomiasis
- Biliary Tract:** See Gallbladder; Liver
- Biochemistry:** See Chemistry
- Bladder, urinary, adenocarcinoma of urachus involving:** 388
- Blastomycosis:** See also Coccidioidosis; Moniliaisis  
reticulum fibers in, 310
- Blood:** See also Hemoglobin and Hemoglobin Compounds; Leukocytes  
colloids; experimental studies in cardiovascular pathology; vibratory lability of plasma colloids in rabbits and in dogs following ingestion of cholesterol, 139
- Diseases:** See Anemia; Leukemia
- groups; diagnosis of erythroblastosis (hemolytic anemia) in macerated fetus, 223

*Blood—Continued*

oxygen; *Plasmodium falciparum* malaria; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating *P. falciparum* infection, 290

pressure, high; hypertension and necrotizing arteritis in rat following renal infarction, 231

*Transfusion*: See Anemia; Blood groups  
Vessels: See Arteries

*Boeck's Sarcoïd*: See Sarcoidosis

*Bones*: See also under names of bones

reticulum fibers of tumors of cartilage and bone, 312

*Booker, W. M.*: Primary carcinoma of liver of dog, 548

*BOOK REVIEWS:*

*A. A. A. S. Research Conference on Cancer: Conference of Papers and Discussions presented at Summer Meeting of Section on Chemistry of American Association for Advancement of Science at Gibson Island, Maryland, July 31-August 4, 1944*, 342

*Bovine Trichomoniasis*; *B. B. Morgan*, 343  
*Journal of History of Medicine and Allied Sciences*, 342

*Kettle's Pathology of Tumors*; *W. G. Barnard and A. H. T. Robb-Smith*, 456

*Physical Chemistry of Cells and Tissues*; *R. Höber and others*, 118

*Preventive Medicine and Public Health*; *W. G. Smillie*, 342

*Pulmonary Edema and Inflammation: Analysis of Processes Involved in Formation and Removal of Pulmonary Transudates and Exudates*; *C. K. Drinker*, 119

*Pulmonary Tuberculosis in Adult: Its Fundamental Aspects*; *M. Pinner*, 221

*Study of Endometriosis, Endosalpingiosis, Endocervicosis, and Peritoneo-Ovarian Sclerosis: Clinical and Pathologic Study*; *J. R. Goodall*, 343

*Synopsis of Pathology*; *W. A. D. Anderson*, 564

*Books received*, 342, 456, 564

*Brain*: See Nervous System; etc.

*Brewer, J. I.*: Chorionic gonadotropin in diagnosis of testicular tumors, 580

*Bright's Disease*: See Nephritis

*Bronchi*, tumors; "alveolar cell tumor" of lung; further evidence of its bronchiolar origin, 175

*Cahill, W. M.*: Morphologic studies of rats deprived of essential amino acids; histidine, 25

*Calcification*: See also under Kidneys  
obstruction of aortic isthmus by calcified thrombus, 63

*Calculus*: See Gallbladder, calculus; etc.

*Cancer*: See also Adenocarcinoma; Choriocarcinoma; Sarcoma; Tumors; and under names of organs and regions; as Bladder; Esophagus; Gallbladder; Liver; Lungs; Stomach; etc.

children's center, 564

epidermoid carcinoma arising in endometrial cyst of ovary, 335

reticulum fibers in adenoma and carcinoma, 315

teratoma of anterior mediastinum in group of military age; 16 cases and review of theories of genesis, 398

*Cannon, W. M.*: Genesis of aortic perforation secondary to carcinoma of esophagus; report of 2 cases, 533

*Carcinoma*: See Cancer

*Cardiovascular Diseases*: See also Heart  
experimental studies in cardiovascular pathology; atheromatosis in dogs following repeated intravenous injections of solutions of hydroxyethylcellulose, 130

experimental studies in cardiovascular pathology; vibratory lability of plasma colloids in rabbits and in dogs following ingestion of cholesterol, 139

syphilis; obstruction of aortic isthmus by calcified thrombus, 63

*Cardiovascular System*: See Arteries; Heart; Vasomotor System

*Cartilage*, reticulum fibers of tumors of, 312  
*Cavanaugh, J. W.*: Structural changes in thyroid glands of patients treated with thiouracil, 155

*Cells*: See also Tissue; etc.

chemical factors and their role in inflammation, 376

*Chagas' Disease*: See Trypanosomiasis

*Chalgren, W. S.*: Tropical diseases; involvement of nervous system, 66

*Chang, T.*: Hepatic abscess complicating atresia of small intestine of newborn infant, 450

*Chemistry*; chemical factors and their role in inflammation, 376

*Chemotherapy*: See under names of diseases and chemotherapeutic agents

*Children*, cancer center, 564

*Cholelithiasis*: See Gallbladder, calculi

*Cholera*, 82

*Cholesterol*; experimental studies in cardiovascular pathology; vibratory lability of plasma colloids in rabbits and in dogs following ingestion of cholesterol, 139

*Choline and Choline Derivatives*; microscopic lesions in acetylcholine shock, 11

*Choriocarcinoma* and rhabdomyosarcoma with teratoma of pineal gland, 552

*Chorionic Gonadotropin*: See Gonadotropins

*Cinchophen*, experimental studies on toxicity and detoxication of, 592

*Cirrhosis*: See under Liver

*Coccidioidosis*, reticulum fibers in, 310

*Colitis, Amebic*: See Amebiasis

*Collins, D. A.*: Changes in central incisors of hypophysectomized female rats after different postoperative periods, 457

*Colloids*: See also Blood, colloids  
structural changes in thyroid glands of patients treated with thiouracil, 155

*Colon*: See Gastrointestinal Tract; Intestines  
Communicable Diseases: See Syphilis; etc.

*Copley Medal*, 120

*Coronary Vessels*; *Plasmodium falciparum* malaria; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating *P. falciparum* infection, 290

*Culbertson, C. G.*: Teratoma of pineal gland with choriocarcinoma and rhabdomyosarcoma, 552

*Curtis, G. M.*: Radiosodium autography in studies of human goitrous thyroid glands, 510

*Cyanol, hemoglobin stain for histologic use based on cyanol-hemoglobin reaction*, 676

*Cysts*: See under names of organs and regions, as Diaphragm; etc.

*Cytology*: See Cells

*Davidsohn, I.*: Joseph McFarland, 338

*Davidson, C. S.*: Acute febrile illness characterized by thrombopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327

*Davis, R. M.*: Morphologic studies of rats deprived of essential amino acids; histidine, 25

*Dengue*, 69

- Dentition: See Teeth
- Dermatitis, Schistosome: See Schistosomiasis
- Detergents; experimental studies in cardiovascular pathology; vibratory lability of plasma colloid in rabbits and in dogs following ingestion of cholesterol, 139
- Diabetes Mellitus; experimental pituitary diabetes of 5 years' duration with glomerulosclerosis, 19  
hepatic and renal necrosis in alloxan diabetes of rabbits, 516
- Diaphragm, primary cystic tumor of, 645
- Diarrhea: See Dysentery
- Diet and Dietetics: See also Vitamins  
morphologic studies of rats deprived of essential amino acids; histidine, 25
- Digestive System: See Gastrointestinal Tract; Intestines; Pancreas; Stomach; etc.
- Dirofilaria: See Filariasis
- Distinguished Service Medal, 220
- Distemiasis; paragonimiasis, 116
- Dogs, primary carcinoma of liver of, 548
- Dohan, F. C.: Experimental pituitary diabetes of 5 years' duration with glomerulosclerosis, 19
- Dublin, W. B.: Reticulum, 299
- Dunn, R. C.: Hemoglobin stain for histologic use based on cyanol-hemoglobin reaction, 676
- Dyes: See Stains and Staining
- Dysentery, Amebic: See Amebiasis  
bacillary, 79
- Edema: See under names of organs and regions, as Lungs; etc.
- Education; fellowships in public health, 454  
postgraduate refresher course for pathologists returning from military service, 120
- Effusions: See Exudates and Transudates
- Emaciation; heart weight; effect of tuberculosis on heart weight, 526
- Embolism: See also Thrombosis  
role of stasis in development of pulmonary infarcts, 319
- Embryology: See Fetus; Teratoma; etc.
- Endamoeba: See Amebiasis
- Endocardium: See Heart
- Enteritis: See Gastrointestinal Tract
- Eosinophils: See also Leukocytes  
transitory pulmonary infiltrations (Loeffler's syndrome) in rabbits, 489
- Epiphysis: See Pineal Gland
- Erskine, C. A.: Analysis of Klippel-Feil syndrome, 269
- Erythroblastosis (hemolytic anemia) in macerated fetus, 223
- Erythrocytes: See Anemia; Blood; Hemoglobin and Hemoglobin Compounds; etc.
- Esophagus, genesis of aortic perforation secondary to carcinoma of esophagus; report of observations in 2 cases, 533
- Ethylene Glycol poisoning with suggestions for its treatment as oxalate poisoning, 631
- Evans, H. M.: Changes in central incisors of hypophysectomized female rats after different postoperative periods, 457
- Exhibits; portable exhibit case, 556
- Exophthalmos: See Goiter, exophthalmic
- Exudates and Transudates; chemical factors and their role in inflammation, 376
- Falk, H. C.: Epidermoid carcinoma arising in endometrial cyst of ovary, 335
- Fat, necrosis; sclerema adiposum neonatorum of both internal and external adipose tissue, 166  
reticulum fibers of tumors of fat cells, 312
- Fell-Klippel Syndrome: See Spine, abnormalities
- Fellowships in public health, 454  
public health laboratory fellowships, 564
- Fertman, M. B.: Radioiodine autography in studies of human goitrous thyroid glands, 510
- Fetus; diagnosis of erythroblastosis (hemolytic anemia) in macerated fetus, 223
- Fever: See also Malaria; Rat Bite Fever; Relapsing Fever; Rocky Mountain Spotted Fever; Typhus; etc.
- acute febrile illness characterized by thrombocytopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327
- chemical factors and their role in inflammation, 376
- leukopenia and inflammation; presence of leukopenic factor in inflammatory exudates, 50
- trench, 78
- Fibroma; reticulum fibers in tumors of fibroblasts, 311
- Filariasis, 112
- Foreign Bodies; reticulum fibers in foreign body giant cell tumors, 312
- Friderichsen-Waterhouse Syndrome: See Adrenals, hemorrhage
- Froats, E. R.: Epidermoid carcinoma arising in endometrial cyst of ovary, 335
- Fungi: See Actinomycosis; Blastomycosis; etc.
- Gallbladder, calculi; relation of cholelithiasis to acute hemorrhagic pancreatitis, 17  
cancer; "alveolar cell tumor" (metastatic) of lung; further evidence of its bronchiolar origin, 175
- Gallstones: See Gallbladder, calculi
- Gastrointestinal Tract: See also Intestines; Stomach; etc.  
enteritis caused by *Salmonella* suispestifer with secondary moniliasis, 540
- Glass, R. L.: Teratoma of pineal gland with choriocarcinoma and rhabdomyosarcoma, 552
- Glomerulonephritis; See Nephritis, glomerular
- Glomerulosclerosis: See Nephritis, glomerular
- Glycosuria: See Urine, sugar
- Goiter: See also Thyroid  
exophthalmic; structural changes in thyroid glands of patients treated with thiouracil, 155  
radioiodine autography in studies of human goitrous thyroid glands, 510
- Golden, A.: Tropical ulcer in Guatemala; pathologic, bacteriologic, mycologic and clinical aspects, 612
- Goldzieher, J. W.: Pituitary lesions accompanying obesity, 203
- Gomori, G.: Distribution of lipase in tissues under normal and under pathologic conditions, 121
- Gonadotropins: See also Pituitary Body; Pituitary Preparations  
chorionic, in diagnosis of testicular tumors, 580
- Gonads: See Ovary; Testes
- Gout, reticulum fibers in, 310
- Gram Stain: See Stains and Staining
- Grants, 341
- Granuloma, Filarial: See Filariasis  
Malignant: See Hodgkin's Disease  
reticulum fibers in several types of, 309
- Graves' Disease: See Golter, exophthalmic
- Growth, Committee on, of National Research Council, 678
- Guatemala, tropical ulcer in; pathologic, bacteriologic, mycologic and clinical aspects, 612
- Gütmann, G. E.: Enteritis caused by *Salmonella* suispestifer with secondary moniliasis, 540

- Hall of Fame, 455  
 Halpert, B.: Structural changes in thyroid glands of patients treated with thiouracil, 155  
 Hartz, P. H.: Development of sebaceous glands from intralobular ducts of parotid gland, 651  
 Human strongyloidiasis with internal auto-infection, 601  
 Occurrence of rheumatic carditis in native population of Curaçao, Netherlands West Indies, 32  
 Health: See Public Health  
 Heart, local tissue reactivity (Schwartzman phenomenon) in heart and femoral artery of rabbit, 565  
*Plasmodium falciparum* malaria; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating *P. falciparum* infection, 290  
 weight, effect of tuberculosis on, 526  
 Heller, E. L.: Ovarian involvement in Hodgkin's disease, 282  
 Struma ovarii, 445  
 Helminths: See Parasites  
 Hemagglutination: See Blood, groups  
 Hemangioma: See Angioma  
 Hemangiosarcoma, splenic; case with lymphatic and vascular metastases, 668  
 Hematin: See Hemoglobin and Hemoglobin Compounds  
 Hematuria: See Nephritis  
 Hemoglobin and Hemoglobin Compounds: See also Anemia; Blood hemoglobin stain for histologic use based on cyanol-hemoglobin reaction, 676  
 Hemolysis: See Anemia; Erythroblastosis; Streptococci  
 Hemolytic Anemia: See Erythroblastosis  
 Hemopericardium: See Pericardium  
 Hemorrhage: See Adrenals  
 Hemostasis, role of stasis in development of pulmonary infarcts, 319  
 Hepatitis: See Jaundice  
 Hepler, O. E.: Experimental nephropathies; glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42  
 Herbut, P. A.: "Alveolar cell tumor" of lung; further evidence of its bronchiolar origin, 175  
 Hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
 Transitory pulmonary infiltrations (Loeffler's syndrome) in rabbits, 489  
 Herzog, A. J.: Parathyroid adenoma, with uremia due to calcification of kidneys, 661  
 Highman, B.: Improved methods for demonstrating amyloid in paraffin sections, 559  
 Histidine; morphologic studies of rats deprived of essential amino acids, 25  
 Hodgkin's Disease, ovarian involvement in, 282  
 Hookworm Infection, 110  
 Hormones: See Adrenal Preparations; Pituitary Preparations; etc.  
 Hueper, W. C.: Experimental studies in cardiovascular pathology; atheromatosis in dogs following repeated intravenous injections of solutions of hydroxyethylcellulose, 130  
 Experimental studies in cardiovascular pathology; vibratory lability of plasma colloids in rabbits and in dogs following ingestion of cholesterol, 139  
 Experimental studies on toxicity and detoxication of cinchophen, 592  
 Hydroxyethylcellulose; experimental studies in cardiovascular pathology; atheromatosis in dogs following repeated intravenous injections of solutions of hydroxyethylcellulose, 130  
 Hypertension: See Blood pressure, high  
 Hyperthyroidism: See under Thyroid  
 Hypophsectomy: See under Pituitary Body  
 Hypophysis: See Pituitary Body  
 Icterus: See Jaundice  
 Inclines: See Teeth  
 Infants: See also Children  
 newborn, hepatic abscess complicating atresia of small intestine of, 450  
 sclerema adiposum neonatorum of both internal and external adipose tissue, 166  
 Infarction: See under Kidneys; Lungs; etc.  
 Infection: See also Viruses; and under names of bacteria, as Streptococci; etc.  
 chemical factors and their role in inflammation, 376  
 Inflammation and leukopenia; presence of leukopenic factor in inflammatory exudates, 50  
 chemical factors and their role in, 376  
 Inguinal Glands: See Lymph Nodes  
 Injuries: See under names of organs and regions  
 Intestines: See also Gastrointestinal Tract  
 Diseases: See Dysentery  
 hepatic abscess complicating atresia of small intestine of newborn infant, 450  
 Parasites: See Amebiasis; Ascariasis; Dysentery; Hookworm Infection; Strongyloidiasis perforation in paratyphoid due to *Salmonella paratyphi B*, 322  
 Iodine and Iodine Compounds; radiiodine autography in studies of human goitrous thyroid glands, 510  
 Irradiation: See under names of diseases and organs  
 Islands of Langerhans: See Pancreas  
 Japanese River Fever: See *Tsutsugamushi Disease*  
 Jaundice, pathologic aspects of acute epidemic hepatitis, with especial reference to early stages; report of 10 cases, including case of spontaneous rupture of spleen and 6 cases of fulminating disease in patients who have been wounded several months previously, 345  
 Jungle Sores: See Ulcers, tropical  
 Kala-Azar: See Leishmaniasis  
 Kean, B. H.: Etiologic concepts and pathologic aspects of albinism, 639  
 Keltz, B. F.: Structural changes in thyroid glands of patients treated with thiouracil, 155  
 Kidneys, Diseases: See Nephritis  
 hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
 hypertension and necrotizing arteritis in rat following renal infarction, 231  
 parathyroid adenoma with uremia due to calcification of, 661  
 renal lesions in portal cirrhosis, 476  
 Kinsey, F. R.: Transitory pulmonary infiltrations (Loeffler's syndrome) in rabbits, 489  
 Klippel-Feil Syndrome: See Spine, abnormalities  
 Laboratories, public health laboratory fellowships, 564  
 Lange, J.: Experimental nephropathies; problem of experimental glomerulonephritis, 185  
 Langerhans' Island: See Pancreas  
 Laskin, M. M.: Microscopic lesions in acetyl-choline shock, 11  
 Leblond, C. P.: Radiiodine autography in studies of human goitrous thyroid glands, 510

- Lederer's Anemia: See Anemia, hemolytic
- Leiomysarcoma involving right ureter, 655
- Leishmaniasis, 101
- Leprosy, 88  
reticulum fibers in, 311
- Lerner, E. M., II: Rapid gram stain for tissue, 674
- Leukemia, reticulum fibers in idiopathic reticuloendothelioses, 311
- Leukocytes: See also Eosinophils; Leukemia; etc.  
chemical factors and their role in inflammation, 376  
count; experimental studies in cardiovascular pathology; atheromatosis in dogs following repeated intravenous injections of solutions of hydroxyethylcellulose, 130  
count; leukopenia and inflammation; presence of leukopenic factor in inflammatory exudates, 50
- Leukopenia: See Leukocytes, count
- Life Insurance Medical Research Fund, 455
- Linn, H. J.: Experimental nephropathies; problem of experimental glomerulonephritis, 185
- Lipase: See also Pancreas, secretion  
distribution in tissues under normal and under pathologic conditions, 121
- Lipoids: See Cholesterol; Fat; etc.
- Lipoma, reticulum fibers of tumors of fat cells, 312
- Liposarcoma, reticulum fibers of tumors of fat cells, 312
- Liver, absorption of scar tissue in experimental nodular cirrhosis with method of visualizing cirrhotic changes, 1  
experimental studies on toxicity and detoxication of cinchophen, 592  
hepatic abscess complicating atresia of small intestines of newborn infant, 450  
hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
primary carcinoma of liver of dog, 548  
renal lesions in portal cirrhosis, 476
- Lober, P.: Parathyroid adenoma with uremia due to calcification of kidneys, 661
- Loeffler Syndrome: See Eosinophils; Lungs, pathology
- Loomis, D.: Hypertension and necrotizing arteritis in rat following renal infarction, 231
- Lukens, F. D. W.: Experimental pituitary diabetes of 5 years' duration with glomerulosclerosis, 19
- Lungs: See also Bronchi; etc.  
"alveolar cell tumor" of; further evidence of its bronchiolar origin, 175  
edema; microscopic lesions in acetylcholine shock, 11  
infarction; role of stasis in development of pulmonary infarcts, 319  
pathology; transitory pulmonary infiltrations (Loeffler's syndrome) in rabbits, 489
- Lymph Nodes: See also Sarcoma; etc.  
splenic hemangiosarcoma; case with lymphatic and vascular metastases, 668
- Lymphangioma: See Angioma
- Lymphatic System: See Lymph Nodes
- Lymphocytes: See Leukocytes
- Lymphogranuloma: See Granuloma
- Hodgkin's: See Hodgkin's Disease
- Schaumann's: See Sarcoidosis
- Lymphoid Tissue: See Lymph Nodes; etc.
- Lymphosarcoma, reticulum fibers in, 313
- McCullough, K.: Epidermoid carcinoma arising in endometrial cyst of ovary, 335
- Madden, E. M.: Sclerema adiposum neonatorum of both internal and external adipose tissue, 166
- Malaria, 92  
Plasmodium falciparum; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating *P. falciparum* infection, 290
- Malformations: See under names of organs and regions, as Spine; etc.
- Marcelle Fleischmann Foundation, 341
- Markowitz, M.: Acute febrile illness characterized by thrombocytopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327
- Martin, R. A.: Absorption of scar tissue in experimental nodular cirrhosis of liver; with method of visualizing cirrhotic changes, 1
- Maun, M. E.: Morphologic studies of rats deprived of essential amino acids; histidine, 25
- Mediastinum, anterior, teratoma in group of military age; 16 cases, and review of theories of genesis, 398
- Medicine, Military: See Military Medicine
- Naval: See Naval Medicine
- Tropical: See Tropical Medicine
- Melanoma, reticulum fibers in, 317
- Melioidosis, 88
- Meningococci, adrenal hemorrhages in meningococcal sepsis, 503
- Menkin, V.: Chemical factors and their role in inflammation, 376
- Leukopenia and inflammation; presence of leukopenic factor in inflammatory exudates, 50
- Mercury bichloride; experimental nephropathies; glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42
- Meritorious Service Plaque, 341
- Merkel, W. C.: Plasmodium falciparum malaria; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating *P. falciparum* infection, 290
- Microfilaria: See Filariasis
- Microorganisms: See Bacteria; etc.
- Military Medicine: See also Naval Medicine  
teratoma of anterior mediastinum in group of military age; 16 cases, and review of theories of genesis, 398
- Milles, G.: Ethylene glycol poisoning with suggestions for its treatment as oxalate poisoning, 631
- Mite Fever: See *Tsutsugamushi* Disease
- Molander, D. W.: Relation of cholelithiasis to acute hemorrhagic pancreatitis, 17
- Moniliasis, secondary, with enteritis caused by *Salmonella* sulpestifer, 540
- Morphea: See Scleroderma
- Morton, D. R.: Primary cystic tumor of diaphragm, 645
- Moses, C.: Role of stasis in development of pulmonary infarcts, 319
- Muscles, reticulum fibers of tumors, noncancerous and cancerous, 312
- Myoma; reticulum fibers of tumors of muscle, noncancerous and cancerous, 312
- Myosarcoma; reticulum fibers of tumors of muscle, noncancerous and cancerous, 312
- National Research Council, Committee on Growth, 678
- Naval Medicine, pathologic aspects of acute epidemic hepatitis, with reference to early stages; report of 10 cases, including case of spontaneous rupture of spleen and 6 cases of fulminating disease in patients who had been wounded several months previously, 345
- Navies: See Naval Medicine
- Neal, M. P.: Fat necrosis studies: effect of feeding lipase-containing vegetable seed on production of fat necrosis, 37

- Necrosin: See Inflammation  
 Necrosis: See also under Fat; Liver; Pancreas  
     hypertension and necrotizing arteritis in rat following renal infarction, 231  
 Neoplasms: See Cancer; Sarcoma; Tumors  
 Neostigmine, microscopic lesions in acetylcholine shock (followed by administration of neostigmine), 11  
 Nephritis: See also Uremia  
     glomerular; experimental nephropathies; problem of experimental glomerulonephritis, 185  
     glomerular; experimental pituitary diabetes of 5 years' duration with glomerulosclerosis, 19  
 Nephropathy: See under Kidneys  
 Nephrosis: See under Kidneys; Nephritis  
 Nerves: See Nervous System  
 Nervous System: reticulum fibers in tumors of nerve origin, 315  
     tropical diseases; involvement of nervous system, 66  
 Netherlands West Indies, occurrence of rheumatic carditis in native population, 32  
 Neurohypophysis: See Pituitary Body  
 Nervi, reticulum fibers in, 317  
 Nixon, C. E.: Adenocarcinoma of urachus involving urinary bladder, 388  
 Nomenclature; adrenal hemorrhages in meningococcic sepsis, 503  
 Obesity, pituitary lesions accompanying, 203  
 OBITUARIES:  
     Evans, Newton, G., 120  
     Flexner, Simon, 678  
     McFarland, Joseph, 338  
     Ravenel, Mazyck Porcher, 220  
     Robertson, Harold Eugene, 455  
     Rosenau, Milton J., 564  
     Ward, Henry B., 120  
 Oriental Sore: See Leishmaniasis  
 Oroya Fever, 78  
 Ovary, epidermoid carcinoma arising in endometrial cyst of, 335  
     ovarian involvement in Hodgkin's disease, 282  
     struma ovarii, 445  
 Oxalates; ethylene glycol poisoning with suggestions for its treatment as oxalate poisoning, 631  
 Padilla B., E.: Tropical ulcer in Guatemala; pathologic, bacteriologic mycologic and clinical aspects, 612  
 Palin, W.: Ovarian involvement in Hodgkin's disease, 282  
 Pancreas, secretion; fat necrosis studies; effect of feeding lipase-containing vegetable seed on production of fat necrosis, 37  
 Pancreatitis, acute hemorrhagic, relation of cholelithiasis to, 17  
 Paraffin: See Specimens  
 Paragonimiasis: See Distomiasis  
 Parasites, animal (helminths), 109  
 Parathyroid adenoma with uremia due to calcification of kidneys, 661  
 Paratyphoid, intestinal perforation due to *Salmonella paratyphi B*, 322  
 Parotid Gland, development of sebaceous glands from intralobular ducts of, 651  
 Pathology, postgraduate refresher course for pathologists returning from military service, 120  
 Peanuts, fat necrosis studies; effect of feeding lipase-containing vegetable seed on production of fat necrosis, 37  
 Pericardium; hemopericardium from pericardial metastatic carcinoma, 550  
     Perkins, E.: Hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
 Phosphatase, experimental nephropathies; glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42  
 Photography, portable exhibit case, 556  
 Pineal Gland, teratoma with choriocarcinoma and rhabdomyosarcoma, 552  
 Pituitary Body, changes in central incisors of hypophysectomized female rats after different postoperative periods, 457  
     diseases; pituitary lesions accompanying obesity, 203  
 Pituitary Preparations, experimental pituitary diabetes of five years' duration with glomerulosclerosis, 19  
 Plague, bubonic, 85  
 Plasmodium: See Malaria  
 Poisons and Poisonings: See under names of specific substances, as Ethylene Glycol  
 Portal Cirrhosis: See Liver  
 Postgraduate Education: See Education  
 Postoloff, A. V.: Genesis of aortic perforation secondary to carcinoma of esophagus; report of observations in 2 cases, 533  
 Potassium dichromate; experimental nephropathies; glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42  
 Potter, E. L.: Diagnosis of erythroblastosis (hemolytic anemia) in macerated fetus, 223  
 Price, S.: Hepatic abscess complicating atresia of small intestines of newborn infant, 450  
 Prizes: See Awards  
 Protozoa, 92  
 Public Health, fellowships in, 454  
     laboratory fellowships, 564  
 Pulmonary Infarcts: See Lungs, Infarction  
 Puppel, I. D.: Radioiodine autography in studies of human goitrous thyroid glands, 510  
 Purpura; acute febrile illness characterized by thrombopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327  
 Q Fever: See Rickettsial Diseases  
 Races: See also Blood groups; etc.  
     etiological concepts and pathologic aspects of albinism, 639  
 Radiations: See under names of various diseases  
 Radioactivity; radioiodine autography in studies of human goitrous thyroid glands, 510  
 Randall, C. C.: Intestinal perforation in paratyphoid due to *Salmonella paratyphi B*, 322  
 Rapoport, A. E.: Adenocarcinoma of urachus involving urinary bladder, 388  
 Rat Bite Fever, 105  
 Relapsing Fever, 103  
 Research, Life Insurance Medical Research Fund, 455  
 surgical endowment for, 678  
 Reticuloendothelial System: See also Anemia; Liver; Spleen; etc.  
     reticulum, 299  
 Reticuloendothelioses: See Leukemia  
 Reticulum: See Reticuloendothelial System  
 Rh Factor: See Blood, groups  
 Rhabdomyosarcoma and choriocarcinoma, with teratoma of pineal gland, 552  
 Rheumatic Fever; occurrence of rheumatic carditis in native population of Curaçao. Netherlands West Indies, 32  
 Rheumatism, reticulum fibers in, 310  
     Acute: See Rheumatic Fever

- Rice, C O Parathyroid adenoma with uremia due to calcification of kidneys, 661
- Rickettsial Diseases, 70 See also Rocky Mountain Spotted Fever, Tsutsugamushi Disease, Typhus
- Q fever, 77
- Rocky Mountain Spotted Fever, 74
- Roentgenotherapy. See under names of diseases
- Rossen, A X Leiomyosarcoma involving right ureter, 655
- Rukstina, G J Hemopericardium from pericardial metastatic carcinoma, 350
- Portable exhibit case, 556
- Russell, T H Leiomyosarcoma involving right ureter, 655
- Salmonella**; enteritis caused by *Salmonella* *uispeistifer* with secondary moniliasis, 340
- Paratyphi B See Paratyphoid
- Sarcoidosis, reticulum fibers in sarcoid of Boeck, 311
- Sarcoma. See also Cancer; Hemangiosarcoma, Leiomyosarcoma, Myosarcoma; Rhabdomyosarcoma; Tumors, etc
- ovarian involvement in Hodgkin's disease, 282
- Schistosomiasis, 113
- Schlumberger, H G Teratoma of anterior mediastinum in group of military age, 16 cases and review of theories of genesis, 390
- Schwartz I Adrenal hemorrhages in meningococcal sepsis, 503
- Sclerema See Scleroderma
- Scleroderma, sclerema adiposum neonatorum of both internal and external adipose tissue, 166
- Sclerosis See Arteriosclerosis, Nephritis
- Scott O B Primary cystic tumor of diaphragm, 645
- Scrub Typhus See Tsutsugamushi Disease
- Sebaceous Glands development from intralobular ducts of parotid gland, 651
- Sensitization See Anaphylaxis and Allergy
- Shock, Acetylcholine See Anaphylaxis and Allergy
- Shwartzman Phenomenon, local tissue reactivity (Shwartzman phenomenon) in heart and femoral artery of rabbit, 565
- Simonds, J P Experimental nephropathies glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42
- Experimental nephropathies, problem of experimental glomerulonephritis 185
- Simpson M E Changes in central incisors of hypophysectomized female rats after different postoperative periods, 457
- Skeleton See under Bones
- Skin, Hemorrhage. See Purpura
- Sleeping Sickness See Trypanosomiasis
- Societies, American Association for Cancer Research, 120, 363
- American Association for Study of Goiter, 474
- American Association of Pathologists and Bacteriologists, 120 474
- American Board of Pathology, 341
- American College of Physicians 120
- American College of Surgeons 454
- Army Institute of Pathology 311
- Association of Pathologists of West Virginia, 120
- Federation of American Societies for Experimental Biology, 120
- Friends of Medical Research, 220
- National Academy of Sciences 363
- Society of American Bacteriologists 220
- Soybeans fat necrosis studies, effect of feeding lipase-containing vegetable seed on production of fat necrosis, 37
- Specimens See also Tissue, sections improved method for demonstrating amyloid in paraffin sections, 559
- Spine, abnormalities, analysis of Klippel-Teil syndrome, 209
- Spleen, hemangiosarcoma; case with lymphatic and vascular metastases, 668
- pathologic aspects of acute epidemic hepatitis, with especial reference to early stages; report of 10 cases, including case of spontaneous rupture of spleen and 6 cases of fulminating disease in patients who had been wounded several months previously, 345
- Specht, L Struma ovarii, 445
- Stains and Staining; hemoglobin stain for histologic use based on cyanol-hemoglobin reaction, 676
- improved methods for demonstrating amyloid in paraffin sections, 559
- pituitary lesions accompanying obesity, 203
- rapid gram stain for tissue, 674
- reticulum, 299
- Stanford, W R Splenic hemangiosarcoma; case with lymphatic and vascular metastases, 668
- Steinberg, B Absorption of scar tissue in experimental nodular cirrhosis of liver, with method of visualizing cirrhotic changes, 1
- Stomach See also Gastrointestinal Tract cancer, adenoacanthoma of stomach, report of case, 213
- Strassmann, G Adenoacanthoma of stomach, report of case, 213
- Streptococci, occurrence of rheumatic carditis in native population of Curaçao, Netherlands West Indies, 32
- Strongyloidiasis, 111
- human, with internal autoinfection, 601
- Struma Ovarii. See Thyroid
- Sugar in Urine See Urine, sugar
- Suprarenal Preparations. See Adrenal Preparations
- Suprarenals See Adrenals
- Syphilis See also under names of organs, regions and diseases
- reticulum fibers in, 309
- Tedeschi, C G Local tissue reactivity (Shwartzman phenomenon) in heart and femoral artery of rabbit, 565
- Teeth, changes in central incisors of hypophysectomized female rats after different postoperative periods, 457
- Teratoma of anterior mediastinum in group of military age, study of 16 cases and review of theories of genesis, 398
- of pineal gland with choriocarcinoma and rhabdomyosarcoma, 552
- Terminology See Nomenclature
- Testes, tumors, chorionic gonadotropin in diagnosis of, 580
- Thiouracil. See Goiter, exophthalmic
- Thorax See Heart, Lungs, Mediastinum, etc
- Thrombopenia. See Purpura
- Thrombosis See also Embolism
- acute febrile illness characterized by thrombocytopenic purpura hemolytic anemia and generalized platelet thrombosis, 327
- obstruction of aortic isthmus by calcified thrombus, 63
- Plasmodium falciparum malaria; coronary and myocardial lesions observed at autopsy in 2 cases of acute fulminating P falciparum infection, 290
- Thyroid. See also Parathyroid
- physiology, structural changes in thyroid glands of patients treated with thiouracil, 153
- radioiodine autography in studies of human goitrous thyroid glands, 510
- struma ovarii, 445

- Tissue: See also Cells  
 absorption of scar tissue in experimental nodular cirrhosis of liver, with method of visualizing cirrhotic changes, 1  
 connective; pituitary lesions accompanying obesity, 203  
 distribution of lipase under normal and under pathologic conditions, 121  
 microscopic lesions in acetylcholine shock, 11  
 section; improved methods for demonstrating amyloid in paraffin sections, 559  
 section; reticulum, 299  
 Staining: See Stains and Staining  
 Translite photographs, portable exhibit case, 556  
 Trench Fever: See Fever, trench  
 Trobaugh, F. E., Jr.: Acute febrile illness characterized by thrombopenic purpura, hemolytic anemia and generalized platelet thrombosis, 327  
 Tropical Medicine, 341. See also under names of tropical diseases, as Leishmaniasis; Ulcers, tropical; etc.  
 tropical diseases; involvement of nervous system, 66.  
 Tropics, diseases in; occurrence of rheumatic carditis in native population of Curaçao, Netherlands West Indies, 32  
 Trypanosomiasis, 96, 99  
 African, 96  
 Tsutsugamushi Disease, 76  
 Tuberculosis: See also under names of various diseases, organs and regions  
 heart weight; effect of tuberculosis on heart weight, 526  
 reticulum fibers in, 309  
 Tucker, H. A.: Etiologic concepts and pathologic aspects of albinism, 639  
 Tumors: See also Adenoma; Angioma; Cancer; Fibroma; Granuloma; Hemangiosarcoma; Leiomyosarcoma; Lipoma; Lipo-sarcoma; Melanoma; Myoma; Myosarcoma; Sarcoma; Teratoma; and under names of organs and regions, as Bronchi; Cartilage; Diaphragm; etc.  
 foreign body giant cell, reticulum fibers in, 312  
 reticulum fibers in neoplasms, 311  
 struma ovarii, 445  
 Typhus, epidemic and endemic, 70  
 Ulcers, Tropical: See also Leishmaniasis  
 tropical, in Guatemala; pathologic, bacteriologic, mycologic and clinical aspects, 612  
 Urachus, adenocarcinoma involving urinary bladder, 388  
 Uranyl nitrate; experimental nephropathies: glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42  
 Uremia; parathyroid adenoma with uremia due to calcification of kidneys, 661  
 Ureters; leiomyosarcoma involving right ureter, 655  
 Urinary Tract: See Kidneys  
 Urine, sugar; experimental nephropathies; glycosuria in dogs poisoned with uranyl nitrate, mercury bichloride and potassium dichromate, 42  
 van der Sar, A.: Occurrence of rheumatic carditis in native population of Curaçao, Netherlands West Indies, 32  
 Vasomotor System: See Arteries; Blood pressure  
 Vegetable Seed: See Peanuts; Soybeans  
 Veins: See Embolism; Thrombosis; etc.  
 Verruga Peruana: See Oroya Fever  
 Vertebra: See Spine  
 Viruses: See also under Yellow Fever; etc.  
 filtrable, 66  
 War: See Military Medicine; Naval Medicine; etc.  
 Waterhouse-Friderichsen Syndrome: See Adrenals, hemorrhage  
 Watson, J. S.: Hepatic and renal necrosis in alloxan diabetes of rabbits, 516  
 Webb, A. C.: Primary carcinoma of liver of dog, 548  
 Weight: See Heart  
 Wood, D. A.: Pathologic aspects of acute epidemic hepatitis, with especial reference to early stages; report of 10 cases, including case of spontaneous rupture of spleen and 6 cases of fulminating diseases in patients who had been wounded several months previously, 345  
 Wuchereria: See Filariasis  
 Yellow Fever, 66  
 Zeek, P. M.: Heart weight; effect of tuberculosis on heart weight, 526  
 Sclerema adiposum neonatorum of both internal and external adipose tissue, 166  
 Zeidman, I.: Intestinal perforation in paratyphoid due to *Salmonella paratyphi B*, 322

